



UNIVERSITY OF LINCOLN

**The frequent exacerbator phenotype of Chronic Obstructive Pulmonary
Disease in pulmonary rehabilitation – assessment of clinical outcomes and
inflammation**

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**THE FREQUENT EXACERBATOR PHENOTYPE OF CHRONIC OBSTRUCTIVE
PULMONARY DISEASE IN PULMONARY REHABILITATION – ASSESSMENT
OF CLINICAL OUTCOMES AND INFLAMMATION**

Alex Robert Jenkins

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Abstract

Chronic obstructive pulmonary disease (COPD) patients who suffer recurrent exacerbations are a recognised clinical phenotype. Pulmonary rehabilitation is considered a cornerstone treatment in the management of COPD. The aim of this thesis was to investigate how frequent exacerbators respond to pulmonary rehabilitation in terms of clinical outcomes and inflammation. Study 1 suggested that frequent exacerbators were less likely to complete pulmonary rehabilitation (44% vs 69%; $p=0.025$), but those who completed experienced clinically important improvements in outcomes. Study 2 demonstrated that fibrinogen concentrations were significantly reduced in both frequent and infrequent exacerbators ($p=0.033$), and total leukocyte ($p=0.018$) and neutrophil ($p=0.018$) counts reduced in frequent exacerbators only upon completion of pulmonary rehabilitation. Study 3 suggested a tendency towards an increase in MKP-1 anti-inflammatory gene expression in response to corticosteroid treatment (2hr, $p=0.060$) in both frequent and infrequent exacerbators upon completion of pulmonary rehabilitation. Study 4 showed that an acute bout of exercise at the beginning of pulmonary rehabilitation induced increases in leukocyte counts ($p=0.002$) and immature neutrophils ($p=0.002$) in both groups. No differences in inflammatory responses to acute exercise were observed with an acute bout of exercise at the end of pulmonary rehabilitation in both groups. Study 5 suggested that frequent exacerbators recorded fewer daily steps ($d=0.3$) and spent less time in light ($d=0.8$) and moderate-to-vigorous activities ($d=0.3$). There were no definitive differences in inflammation between frequent and infrequent exacerbators. Overall, this thesis shows that pulmonary rehabilitation should be encouraged in frequent exacerbators as they stand to have significant improvements in clinical outcomes and resolution of inflammation.

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List of Abbreviations

~ - approximately

µg – microgram

µl – microlitre

6MWT – 6-minute walk test

ADAM17 – ADAM metallopeptidase domain 17

ANOVA – Analysis of Variance

ATS – American Thoracic Society

BD – Becton Dickinson

BMI – Body mass index

BTS – British Thoracic Society

BV – Bright violet

cAMP – Cyclic adenosine monophosphate

CAT – COPD assessment tool

CCI – Charlson Comorbidity Index

CD – Cluster of differentiation

cDNA – Complimentary deoxyribonucleic acid

cGMP – Cyclic guanosine monophosphate

CCRS – Countywide Community Respiratory Service

COPD – Chronic Obstructive Pulmonary Disease

CoS – College of Science

CRP – C-reactive protein

CRQ – Chronic respiratory questionnaire

CXCL – Chemokine ligand

CXCR – Chemokine receptor

DOI – Declaration of Interest

ECLIPSE – Evaluation of COPD longitudinally to identify predictive surrogate endpoints

ELISA – Enzyme-linked immunosorbent assays

ERK – Extracellular signal-regulated kinases

ERS – European Respiratory Society

E-RS™ – Evaluating Respiratory Symptoms

ESWT – Endurance shuttle walk test

EXACT® – Exacerbation of COPD tool

FACS – Fluorescence-activated cell sorting

FEV₁ – Forced expiratory volume in one second

FVC – Forced vital capacity

GAPDH – Glyceraldehyde 3-phosphate dehydrogenase

gDNA – Genomic deoxyribonucleic acid

GILZ – Glucocorticoid-induced leucine zipper

GOLD – Global Initiative for Chronic Obstructive Lung Disease

GP – General Practitioner

HADS – Hospital anxiety and depression scale

HDAC2 – Histone deacetylase 2

hr – Hour

HRA – Health Research Authority

ICS – Inhaled corticosteroids

IL – Interleukin

ISWT – Incremental shuttle walk test

K₃EDTA – Ethylenediaminetetraacetic acid

kg – Kilogram

LABA – Long-acting beta-agonist

LAMA – Long-acting muscarinic antagonists

LCHS – Lincolnshire Community Health Services

MAPK – mitogen-activated protein kinases

MCID – Minimally clinically important difference

METS – Metabolic equivalent of task

MFI – Median fluorescent intensity

mg/dL – milligram/decilitre

mg/L – milligram/litre

min – minute

MKP-1 – Mitogen-activated kinase phosphatase-1

ml – millilitre

MMP – Matrix metalloproteinase

mMRC – modified Medical Research Council

mRNA – messenger RNA

n – Number

NHS – National Health Service

NICE – National Institute for Health and Care Excellence

NT – Not-treated

PBMC – Peripheral blood mononuclear cell

PCR – Polymerase chain reaction

PDE4 – Phosphodiesterase type 4

REC – Research Ethics Committee

Rehab – Rehabilitation

RGS2 – regulator of G-protein signalling 2

RNA – Ribonucleic acid

ROS – Reactive oxygen species

RPMI – Roswell Park Memorial Institute

SABA – Short-acting beta-agonist

SAMA – Short-acting muscarinic antagonists

SD – Standard deviation

SGRQ – St. Georges respiratory questionnaire

SPSS – Statistical Package for Social Sciences

TNF- α – Tumour necrosis factor-alpha

UK – United Kingdom

URTI – Upper respiratory tract infection

USA – United States of America

VILO – Variable input linear output

W – Water

List of Publications from this Thesis

Full article

JENKINS, A. R., GOWLER, H., CURTIS, F., HOLDEN, N. S., BRIDLE, C. & JONES, A. W. 2018. Efficacy of supervised maintenance exercise following pulmonary rehabilitation on health care use: a systematic review and meta-analysis. *International journal of chronic obstructive pulmonary disease*, 13, 257-273.

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Conference Proceedings

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- JENKINS, A. R., HOLDEN, N. S. & JONES, A. W. 2017. The effect of exercise training on inflammatory responses to acute exercise in patients with COPD: preliminary findings. *Physiological Society: Future Physiology Conference (University of Leeds)*
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- JENKINS, A. R., HOLDEN, N. S. & JONES, A. W. 2018. The effect of pulmonary rehabilitation on markers of neutrophil activation in COPD. *Midlands Innovation Flow Cytometry Meeting (University of Birmingham)*
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- JENKINS, A. R., HOLDEN, N. S. & JONES, A. W. 2018. The effects of pulmonary rehabilitation on blood neutrophil activation and functional subsets in COPD. *United Kingdom Society for Exercise Immunology (Loughborough University)*
- JENKINS, A. R., HOLDEN, N. S. & JONES, A. W. 2018. The effects of pulmonary rehabilitation on markers of systemic inflammation in COPD: preliminary findings. *British Association for Lung Research (University of Birmingham)*
- JENKINS, A. R., GIBBONS, L., HOLDEN, N. S. & JONES, A. W. 2018. Responses of exacerbation phenotypes of COPD to community pulmonary rehabilitation. *East Midlands Thoracic Society Meeting (Loughborough University)*

1 Chapter ONE

Literature Review

1.1 Definition and burden of COPD

Chronic Obstructive Pulmonary Disease (COPD) is a common, preventable and treatable disease characterised by persistent respiratory symptoms and largely irreversible and progressive airflow limitation that is due to airway and/or alveolar abnormalities induced by exposure to noxious stimuli (Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2019).

COPD is a leading cause of morbidity and mortality worldwide that triggers an economic and social burden that is both substantial and increasing (Lozano et al., 2012). There are estimated to be 1.2 million diagnosed COPD patients in the United Kingdom (UK), however, estimates believe that there are over 3 million people living with the condition (National Institute for Health and Care Excellence (NICE), 2018). COPD results in approximately 23,000 deaths a year in the UK (Department of Health, 2012a), and represents a significant expense (annual cost to the National Health Service (NHS) of £1.9 billion) as the second largest cause of emergency hospital admissions (British Lung Foundation, 2017).

1.2 COPD disease development

1.2.1 Risk factors

Historically, the most common risk factor for COPD has been tobacco smoking (Quint & Wedzicha, 2007). Prevalence of COPD is higher in smokers and ex-smokers when compared to non-smokers (Fukuchi et al., 2004; Halbert et al., 2006; Menezes et al., 2005). Chronic exposure to an irritant, such as tobacco smoke, amplifies the normal inflammatory response in the lung (Rabe et al., 2007), causing

a progressive decline in lung function and increase in airflow limitation (de Godoy et al., 1996; Fabbri & Rabe, 2007; Hoenderdos & Condliffe, 2013).

It has been estimated that at least 25% of smokers will develop clinically significant COPD (Lokke et al., 2006). However, research has shown that 25-45% of patients with chronic airflow limitation have never smoked highlighting other potential causal components (Eisner et al., 2010; Salvi & Barnes, 2009). Causal components such as; indoor air pollution, passive smoking, exposure to occupational dusts and/or chemicals have been identified and are increasingly considered to be important contributors to the pathogenesis of COPD (GOLD, 2019). Nevertheless, due to the nature of COPD development in smokers and non-smokers, it has long been advocated that genetic components are also important factors in COPD development (Smith & Harrison, 1997). A key risk factor in patients who have never smoked is a severe hereditary deficiency of α_1 -antitrypsin (Stoller & Aboussouan, 2005). α_1 -antitrypsin has a primary role of protecting tissues (e.g. lung and the airways) from the damaging effects of proteases derived from inflammatory cells, in particular neutrophil elastase, which is considered to be actively involved in destruction of lung tissue (e.g. elastin breakdown) and disease pathophysiology (Hoenderdos & Condliffe, 2012). Recent evidence has proposed a further 97 genetic signals which may be involved in the pathogenesis of COPD, reflecting a complex genetic component which stems beyond α_1 -antitrypsin alone (Wain et al., 2017). Such genetic components may become targets of future therapies to prevent chronic airflow limitation (Wain et al., 2017).

1.2.2 Pathology

To be able to produce therapies that target the management of COPD, it is important to understand the underlying pathophysiology. Chronic airflow limitation in COPD is caused by a mixture of small airways disease (obstructive bronchiolitis),

mucus hypersecretion (chronic bronchitis) and parenchymal destruction (emphysema) (GOLD, 2019). These pathological changes are underpinned by chronic inflammatory processes including increased number of inflammatory cells within the lung and structural changes due to exposure to inhaled tobacco smoke and/or irritants (Lee, Taneja & Vassallo, 2012). One identified progressive process is airway remodelling causing a loss of elasticity in lung tissue, especially the recoiling component, due to emphysematous destruction (Decramer, Janssens & Miravittles, 2012). This emphysematous destruction and repeated tissue damage can lead to the development of fibrotic tissue reducing the expansion capacity of the lungs and the ability of the lungs to fully clear on exhalation (Ferguson, 2006). The risk of dynamic hyperinflation, a severe form of breathlessness for COPD patients, increases as a result of gas trapping that follows the combination of loss of elasticity within the alveoli tissue and narrowing of the airways (Ferguson, 2006). Furthermore, structural changes including the loss of; respiratory alveoli, bronchioles and alveolar ducts have been seen to occur in COPD leading to mucociliary dysfunction and reduced mucous clearance (Quint & Wedzicha, 2007). Typically, the inflammation and structural changes increase with COPD severity and appear to persist within the lung even in the absence of further exposure to risk factors.

1.2.3 Inflammatory cells and mediators

COPD is underpinned by chronic inflammation in both the systemic and airway compartments (Rovina, Koutsoukou & Koulouris, 2013; Stockley, Mannino & Barnes, 2009). A complex network of immune pathways, including cells and signalling proteins are involved in the inflammatory processes resulting in disease pathogenesis and progression.

1.2.3.1 Macrophages

Macrophages are important cells in the immune response as they are involved in the process of efferocytosis whereby apoptotic cells are removed (Mosser & Edwards, 2008). In COPD, macrophage numbers are increased in the lungs and are believed to orchestrate inflammation through the release of chemokines that attract many other immune cells including neutrophils (Barnes, 2004; Barnes, 2008). The continued elevated presence of macrophages in the lungs may be partly explained by the defective nature of these cells in COPD populations to engulf apoptotic cells, and hence failure to adequately resolve inflammation (Hodge et al., 2003).

1.2.3.2 Epithelial cells

Epithelial cells play an important role in protecting the body from noxious substances as they act as a defensive barrier between the external environment and the internal milieu (Gao et al., 2015). Airway epithelial cells are important in the defence of the airways as they produce mucus from goblet cells and secrete antioxidants (Barnes, 2016). Epithelial cells are important in the pathogenesis of COPD as exposure to cigarette smoke, or other irritants, triggers these cells to release inflammatory cytokines and chemotactic factors attracting inflammatory cells to the lungs (Barnes 2008). Epithelial cells also play a role in the fibrosis of small airways due to this pro-inflammatory response to noxious stimuli combined with a loss of cilia resulting in irreversible narrowing of the airways (Hogg et al., 2004). Alongside macrophages, epithelial cells can release proteases such as matrix metalloproteinase-9 (MMP-9), which causes elastin degradation contributing towards emphysema (Barnes, 2008). Acute and/or chronic exposure of these cells to noxious material increases the pro-inflammatory response (e.g. increases in

MMP-9) shifting the balance between pro- and anti-inflammatory in favour of pro-inflammatory mediators (Barnes, 2016).

1.2.3.3 Neutrophils

Neutrophils are the most dominant leukocyte in the circulation, accounting for 50-60% of blood leukocytes and are a part of the granulocyte family. They play a key role in the innate immune response, acting as the first line of defence against microbial invasion or tissue debris removal (Bouman et al., 2004; Malm, Lenkei & Sjodin, 1999; Nieman, 2001). Neutrophil recruitment to sites of inflammation is usually central to bacterial clearance and inflammation resolution (Hoenderdos & Condliffe, 2013).

Whilst recruitment of neutrophils can be seen to occur in healthy lungs, this is amplified in COPD whereby neutrophils are recognised to be the major immune cell type in pathogenesis of the disease (Quint & Wedzicha, 2007). Elevated levels of neutrophils have been observed in both the lung and systemic compartments in patients with COPD (Hoenderdos & Condliffe, 2013). Evidence has supported the importance of neutrophils in COPD as elevated neutrophil counts in the lung have been linked to the: degree of airway obstruction (Pilette et al., 2007), development and severity of emphysema (Parr et al., 2006), and rate of lung function decline (Donaldson et al., 2005a). The amplified neutrophil response in COPD shifts the balance between protease/antiprotease in favour of lung tissue destruction (Quint & Wedzicha, 2007). Neutrophils are prominent in COPD due to the chronic inflammatory responses characterised by elevated macrophage numbers in the lung releasing chemokines to recruit neutrophils (Barnes, 2004; Barnes, 2008). Exposure to airway irritants also triggers an inflammatory response attracting neutrophils to the lungs (Barnes, 2016). The chronic accumulation of neutrophils in the airways can also lead to tissue damage due to production of reactive oxygen

species (ROS) exceeding antioxidant defences (Barnes, 2016). This state of oxidative stress has also been proposed to impair antiprotease defences (Cavarra et al., 2001), further accelerating the breakdown of elastin in the lung parenchyma (Barnes, 2016).

1.2.3.4 Eosinophils

Eosinophils, like neutrophils, are part of the granulocyte family. Eosinophils are responsible for triggering inflammatory responses in allergic disorders such as asthma (Walford & Doherty, 2014). Eosinophilic inflammation is more apparent in asthma compared to the neutrophilic inflammation observed in COPD (Barnes, 2008). However, eosinophils can be observed to be elevated in COPD and are seen to play an increasing role in disease progression and treatment, particularly in patients with asthmatic features (Tashkin & Wechsler, 2018).

1.2.3.5 T cells

T cells are another important cell type which have been shown to play a role in airway remodelling (Kim & Criner, 2013). T cells have been observed to be elevated in patients with COPD, especially patients with more severe airflow limitation (Saetta et al., 1998). T cells have also been shown to orchestrate inflammatory processes and accumulate in the lung of COPD patients resulting in structural damage (Barnes, 2008).

1.2.3.6 Cytokines and chemokines

Inflammatory mediators including cytokines and chemokines have also been implicated in the development of COPD (Donnelly & Barnes, 2006). Cytokines and chemokines derived from inflammatory cells in the lung interact with each other in a complex manner to perpetuate the chronic inflammatory response, via the recruitment of further inflammatory cells (Barnes, 2014).

Cytokines are small secreted proteins released by cells which interact with other cells (e.g. signalling) (Zhang & An, 2007). Several cytokines have been implicated in COPD as mediators of chronic inflammation (Barnes, 2008; Barnes, 2009). Tumour necrosis factor alpha (TNF- α) is one of these cytokines increased in the airways of COPD patients (Mukhopadhyay, Hoidal & Mukherjee, 2006), and is commonly produced by macrophages and monocytes during acute inflammation causing a diverse range of signalling events (Idriss & Naismith, 2000). Interleukin-6 (IL-6) is another cytokine which stimulates the acute phase response in the event of tissue injury or infection (Tanaka, Narazaki & Kishimoto, 2014). IL-6 levels are significantly elevated in the airways and circulation of COPD patients playing a role in the amplification of inflammation underpinning the disease (de Moraes et al., 2014).

Chemokines are proteins with the ability to stimulate the recruitment and activation of leukocytes (Groves & Jiang, 1995). Several chemokines have also been implicated in the development of COPD (Barnes, 2014). Chemokine ligand-8 (CXCL8), also known as IL-8, is one of the chemokines implicated in COPD as characterised by increased concentrations in sputum (Aaron et al., 2001; Keatings et al., 1996), and is secreted from macrophages, T cells, epithelial cells, and neutrophils (Barnes, 2014). CXCL8 induces chemotaxis of neutrophils towards sites of inflammation (Russo et al., 2014). CXCL8 is believed to be a key chemokine in the self-perpetuating notion of chronic persistent inflammation in both the airways and circulation of COPD (Traves et al., 2002). CXCL8 plays a role in neutrophil activation via chemokine receptor-1 (CXCR1) and is chemotactic for neutrophils via CXCR2, which can also be activated by CXCL1 (Barnes, 2014). Like CXCL8, concentrations of CXCL1 have been also observed to be increased in the airways of COPD patients (Traves et al., 2002).

Overall, the aforementioned cells and mediators underpin the integral role chronic inflammation, both systemically and in the airways, plays in the pathophysiology of COPD (Di Stefano et al., 1998; Hogg et al., 2004). Current understanding demonstrates that inflammatory cells and mediators play a key role in the development of airflow limitation and increasingly in disease symptomology (Rennard, 2007).

1.3 COPD disease activity

1.3.1 Symptoms

A clinical diagnosis of COPD is considered in any individual who has dyspnoea, chronic cough or frequent sputum production, and an underlying history of chronic exposure to risk factors for COPD (NICE, 2018).

1.3.1.1 Dyspnoea

The most common symptom in COPD is chronic and progressive dyspnoea. Chronic dyspnoea is a major cause of disability and anxiety (Miravittles et al., 2014), whereby patients have often described the sensation of dyspnoea as a starvation of air, periods of gasping, chest heaviness, and an increased effort required to breathe (Elliott et al., 1991). The progressive nature of dyspnoea in COPD occurs because of structural changes with disease progression, e.g. narrowing of the airways (Quint & Wedzicha 2007). Muscle deconditioning, commonly observed in COPD, also contributes to the increased requirement of ventilation during exertion leading to heightened symptoms of dyspnoea (Roig et al., 2011). However, there are other neural and perceptual factors which can influence the sensation of dyspnoea in COPD (O'Donnell et al., 2007). For example, disruption of the respiratory feedback pathways to the brain (O'Donnell et al., 2007). This illustrates the multicomponent factors associated with assessing dyspnoea in COPD.

1.3.1.2 Cough

Persistent coughing is also a characteristic of COPD and is often the first symptom to present. Coughing can often be disregarded as a symptom as it is often perceived as an expected consequence of exposure to noxious material (GOLD, 2019). During the early stages of COPD, coughing may be intermittent, but with development, coughing can be present every day in varying degrees. The causes of cough in COPD are poorly understood but are in part believed to be as a result of airway inflammation (Smith & Woodcock, 2006). It has also been suggested that mucus secretion is also a component in coughing in COPD (Joos et al., 2003). Chronic coughing in COPD can be productive or unproductive in terms of sputum production (Cho et al., 2016). Although cough is a major symptom of COPD, airflow limitation can develop in the absence of a cough and sputum production.

1.3.1.3 Sputum

Frequent sputum production is present in up to 30% of COPD patients (GOLD, 2019). Sputum production is common in COPD due to the mucus hypersecretion that comes with disease development (GOLD, 2019). Production of sputum can be intermittent in periods of bacterial infections with expectorated sputum used by clinicians to identify causative pathogens and guide treatment (Allinson et al., 2016).

1.3.1.4 Chest

Chest tightness and wheezing are also symptoms commonly experienced by COPD patients. These symptoms can vary across the timespan of a single day, and severity is sensitive to change across days. However, when considering respiratory symptoms such as chest tightness and wheezing, it is important to consider that other factors such as comorbidities can contribute to the severity of these symptoms (Lee et al., 2017).

1.3.2 Systemic manifestations and co-morbidities

Although thorough assessment of pulmonary manifestations and respiratory symptoms are essential for diagnosis, the disease stems beyond the pulmonary compartment with systemic effects associated with the lung disease triggering the development of comorbidities (Decramer et al., 2008; Fabbri et al., 2008). These comorbidities can independently affect health status and outcomes, increasing the risk of health care resource use (Mannino et al., 2008; Sin et al., 2006).

Evidence has found that up to 50-60% of COPD patients can present with at least one comorbidity (Crisafulli et al., 2008; Crisafulli et al., 2010). Some of the most common comorbidities in COPD include: heart failure, diabetes, osteoporosis, malnutrition and anxiety disorders (Cavailles et al., 2013). However, such is the diversity of comorbidities being presented with COPD; it is important to assess for appropriate individualised treatment plans (Cavailles et al., 2013). Comorbidities alongside COPD can have a negative impact on clinical outcomes and may contribute to disease progression and morbidity (Cavailles et al., 2013).

However, whether comorbidities are a direct result of COPD progression remains unclear. Some comorbidities have been suggested to be directly caused by COPD, for example, pulmonary artery disease and malnutrition. Other comorbidities such as anxiety, depression, obesity, osteoporosis, diabetes and sleep disturbance have not been directly identified to be a result of COPD (Cavailles et al., 2013). The fact that smoking is also a risk factor for many of these comorbidities makes it difficult to draw conclusions about relationships (Chatila et al., 2008). However, the majority of these extrapulmonary manifestations have a common ground of chronic systemic inflammation (Cavailles et al., 2013), and may contribute towards the exacerbation of respiratory symptoms (Hyeon Jeong et al., 2016).

1.3.2.1 Systemic inflammation

Systemic inflammation has been suggested to play a key role in the development of systemic manifestations of COPD (Vestbo, 2007). The presence of systemic inflammation in COPD has previously been confirmed by elevated levels of markers of inflammation including C-reactive protein (CRP), IL-6, fibrinogen, and leukocytes (Gan et al., 2004).

Fibrinogen and CRP are acute phase proteins found in circulating plasma which increase in response to inflammation (Jain, Gautam & Naseem, 2011). The acute phase response is a systemic response to local or systemic disorders affecting homeostasis commonly caused by infection or tissue injury (Gordon & Koy, 1985; Grus et al., 1999). As part of the acute phase response, pro-inflammatory cytokines are released and inflammatory cells are activated leading to further production of cytokines and other inflammatory mediators (Gruys et al., 2005; Jain, Gautam & Naseem, 2011). Fibrinogen is an acute phase protein which is converted into fibrin via the action of thrombin which forms a fibrous mesh that impedes blood flow during the clotting process (Duvoix et al., 2013). Fibrinogen concentration increases in response to increased IL-6 production and plays an important role in maintaining haemostasis (Gabay & Kushner, 1999; Mackiewicz et al., 1991). Fibrinogen is a commonly used marker to assess for cardiovascular disease risk (Stec et al., 2000), but is becoming a more prominent marker in COPD. In COPD, fibrinogen levels are elevated and considered to be an 'ideal' blood biomarker for the existence of systemic inflammation (Duvoix et al., 2013). CRP is also an acute phase protein which is increased in the plasma in response to infection via the action of IL-6 (Ridker, 2016). CRP is believed to play a key role in the innate immune system by binding to Fc receptors resulting in the production of proinflammatory cytokines to amplify the inflammatory response (du Clos, 2000). CRP levels are raised in COPD

patients and are associated with poorer lung function and exercise capacity (Aksu et al., 2013). Elevated CRP levels have also been suggested to be indicative of low-grade systemic inflammation in COPD (Mannino, Ford & Redd, 2003; Sin & Man, 2003).

The identification of these biomarkers in COPD have been associated with negative outcomes. For example, heightened persistent systemic inflammation has been associated with a higher incidence of exacerbations and an accelerated decline in lung function demonstrating the importance of assessing these biomarkers in COPD (Agusti et al., 2012; Hurst et al., 2006).

1.3.3 Exacerbations of COPD

1.3.3.1 Defining exacerbations

Exacerbations are defined as events in the natural course of COPD, characterised by an acute worsening of respiratory symptoms beyond that of day-to-day variation that results in additional therapy (GOLD, 2019). Exacerbations of COPD are important events in COPD as they: accelerate lung function decline (Donaldson et al., 2002; Kanner, Anthonisen & Connett, 2001), negatively impact on quality of life (Kessler et al., 2006 Spencer et al., 2004), accelerate physical activity decline (Demeyer et al., 2018), and increase respiratory symptoms (Seemungal et al., 2000).

The severity of exacerbations is variable. Some patients present with mild to moderate COPD exacerbations explained by worsening daily symptoms requiring treatment with antibiotics and oral corticosteroids, whilst others may display with more severe exacerbations requiring hospitalisation and in some instances prolonged ventilator support as a consequence of acute respiratory failure (Bafadhel et al., 2011; Seemungal et al., 2000; Spruit et al., 2016a). Anthonisen et al. (1987) have previously defined acute exacerbations based on the following

clinical criteria: increased sputum volume, increased sputum purulence, and increased dyspnoea. Using these criteria, Anthonisen et al. (1987) categorised exacerbations into 3 categories: mild (one symptom present plus one of the following: upper respiratory tract infection (URTI) within 5 days, increased wheezing or cough, fever without an obvious source, 20% increase in respiratory rate, heart rate raised above baseline), moderate (any two symptoms present), and severe (all three symptoms present). COPD patients usually recover from exacerbations within 7-10 days of onset, but in some cases, this can be prolonged with approximately 20% of exacerbations not reaching a recovered pre-exacerbation state 8 weeks after onset (Seemungal et al., 2000).

The Anthonisen et al. (1987) definition of exacerbations has been developed over the years. The categorisation of exacerbations as mild, moderate or severe are still used but have been adapted based on treatments required (e.g. use of short-acting beta₂-adrenoceptor agonist (SABA), oral corticosteroids/antibiotics, hospitalisation). This is considered to provide a more tailored categorisation of exacerbations with the severity of exacerbations also being found to relate to a cause (GOLD, 2019).

1.3.3.2 Causes of exacerbations

Exacerbations are the most prominent cause of hospitalisation in COPD accounting for 50-75% of healthcare costs related to COPD (Donaire-Gonzalez et al., 2015), with 60% of these exacerbations caused by respiratory (viral or bacterial) infection (Barnes, 2000).

Bacterial exacerbations account for a substantial amount of COPD exacerbations with increased colonisation by pathogenic strains being a suggested cause of an exacerbation (Sethi, 2004). One of the main characteristics of bacterial exacerbations is the presence of purulent sputum reflecting an increase in

inflammatory mediators which can be seen in mild, moderate and severe exacerbations (Brusse-Keizer et al., 2009; Soler et al., 2012). In bacterial infections, *H. influenza*, *S. pneumoniae*, and *M. catarrhalis* are the most prominent pathogens in the lower airways of patients with exacerbations of COPD (Blasi et al., 2002; Monso et al., 1995; Pela et al., 1998; Woodhead et al., 2011).

Viral exacerbations also account for a significant total of exacerbations of COPD (Wedzicha & Donaldson, 2003). Viral exacerbations are more likely to lead to severe exacerbations (e.g. hospitalisation) and greater level of airway inflammation (Wedzicha & Donaldson, 2003). Common viruses resulting in respiratory tract infections in COPD include; rhinovirus, coronavirus, influenza A and B (Wedzicha & Donaldson, 2003).

It is also important to note that changes in the environment can cause exacerbations including exposure to outdoor pollution/dusts and changes in temperature (sudden changes from hot to cold or vice versa) (Sapey & Stockley, 2006). There are also several non-physical causes of hospital admissions for exacerbations of COPD to consider. Heightened levels of anxiety and depression have been shown to be a mainstay during exacerbations and are often an underlying cause of hospital admission (Kessler et al., 2006; Laurin et al., 2011; Ng et al., 2007; Spruit et al., 2016a). Other non-physical factors such as poor coping skills, social isolation and poor resilience have been suggested to be other underlying causal factors for hospitalised exacerbations (Fitzgerald & Poureslami, 2014; Kessler et al., 2006; Spruit et al., 2016a).

1.3.3.3 Exacerbations and Inflammation

Exacerbations are usually inflammatory events with increases in several markers of inflammation being seen within the airways and systemically (Perera et al., 2007). Inflammatory markers such as CRP, fibrinogen, IL-6, TNF- α , and leukocytes have

been seen to be increased in the event of an exacerbation (Johansson et al., 2014; Markoulaki et al., 2011).

CRP, fibrinogen, and neutrophil counts are observed to be elevated in a stable state in COPD (Aksu et al., 2013; Duvoix et al., 2013; Hoenderdos & Condliffe, 2013), but during exacerbations, these markers of systemic inflammation are elevated further (Hurst et al., 2006; Wedzicha et al., 2000). Evidence has suggested that during exacerbations circulating neutrophils: are dysregulated through up-regulated expression of pro-inflammatory related genes, produce more ROS and elastase, and have an enhanced expression of cellular adhesion molecules (Fujimoto et al., 2005; Noguera et al., 1998; Oudijk et al., 2005; Vaitkus et al., 2013). This recruitment of neutrophils to sites of inflammation is mediated by intra- and extra-cellular signalling. Blood neutrophils are identified by cell surface expression of CD16 & CD45 and undergo adhesion, mediated by CD62L, to the endothelial cells before rolling down the endothelium. During this rolling process cell activation occurs, characterised by increased CD11b and CD66b expression, before arresting on the endothelium and strengthening adhesion. This stronger adhesion leads to cell spreading before neutrophil extravasation into tissue (Ley et al., 2007) (Figure 1.1).

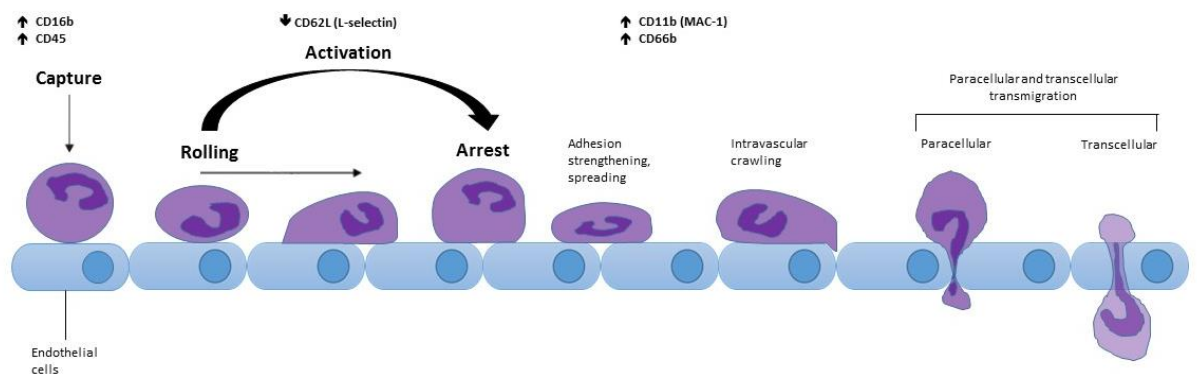


Figure 1.1 Process of neutrophil adhesion and activation cascade. Neutrophils are identified by high expression of CD16 (FcγRIIIb) and CD45. Adhesion cascade comprises of three steps: rolling (mediated by selectins (e.g. CD62L)), activation

(mediated by chemokines), and arrest (mediated by integrins). CD62L (L-selectin) is shed during the process of neutrophil activation, with an upregulation in CD11b (MAC-1) and CD66b as the process of activation progresses during migration. Adapted from Ley et al., (2007).

Following exacerbation, blood parameters as well as lung function are expected to eventually return to pre-exacerbation levels, however, on rare occasions, persistent changes can be seen (Donaldson et al., 2015; Donaldson & Wedzicha, 2017). Measuring these cellular responses is important as patients who present with persistent inflammation tend to have more severe COPD and are more at risk of mortality and suffering future exacerbations compared to those without systemic inflammation (Agusti et al., 2012). However, it is not yet known whether persistent systemic inflammation is a cause or effect of increased exacerbation risk (Agusti et al., 2012).

1.4 COPD disease severity

1.4.1 Airflow limitation

In order to confirm the presence and severity of airflow limitation (and hence make the diagnosis of COPD) spirometry is performed in individuals with dyspnoea, chronic cough or sputum production and a history of exposure to risk factors. A clinical diagnosis of COPD in such patients is based on a post-bronchodilator spirometry reading of Forced Expiratory Volume in one second (FEV_1)/Forced Vital Capacity (FVC) <0.70 confirming the presence of airflow limitation (Gruffydd-Jones, 2012). The GOLD consensus report on the global strategy for the diagnosis, management and prevention of COPD in 2001 (GOLD, 2001), categorised COPD in four stages based on severity of airflow obstruction: stage 1 (mild, $FEV_1 \geq 80\%$ of predicted), stage 2 (moderate, FEV_1 50-80% of predicted), stage 3 (severe, FEV_1 30-50% of predicted), and stage 4 (very severe, $FEV_1 <30\%$ of predicted).

Despite the widespread use of spirometry for the diagnosis of COPD, there are limitations with this method. There is a weak correlation between FEV₁, symptom severity, and patient health status (Jones, 2009), and spirometry provides little information on disease activity (Paone et al., 2016). With this in mind, more recent international guidelines have incorporated symptom measurements and exacerbation history to provide a better overview of disease severity (GOLD, 2019).

1.4.2 Symptoms monitoring and assessment

The assessment of respiratory symptoms is important in COPD management. Respiratory symptoms are often associated with functional capacity and infectious events (Sethi, 2010; Troosters et al., 2013).

In practice, the modified Medical Research Council (mMRC) scale, Chronic Respiratory Questionnaire (CRQ), St. Georges Respiratory Questionnaire (SGRQ), or the COPD Assessment Tool (CAT) are commonly used pre- and post-intervention to assess symptomatic changes (Lacasse et al., 2006). These assessments of symptoms can be broadly used to carry out a measurement of overall health status in response to treatment or with an aim to monitor exacerbation risk.

The mMRC scale is a classical measure of breathlessness which grades patients from 0-4 depending on the self-reporting of functional limitation due to breathlessness (Fletcher, 1960). This tool has been shown to correlate well with other measures of health status (Bestall et al., 1999). The CRQ, SGRQ and CAT are widely used and cover specific domains including: dyspnoea, emotion, fatigue, mastery, activity, impact, cough, sputum, chest tightness, and sleep (Dodd et al., 2011; Ferrer et al., 2002; Williams et al., 2001). These tools build on the previous use of the mMRC scale to understand overall symptoms rather than focussing on dyspnoea alone. However, these tools are limited to measuring symptoms at

selected timepoints providing a general overview failing to capture daily symptoms which have been seen to fluctuate in COPD (Roche, Chavannes & Miravitlles, 2013).

The large and increasing burden of COPD exacerbations has led to interest in identifying fluctuations in symptoms, specifically capturing clinically relevant acute worsening of respiratory symptoms by daily monitoring/symptom diaries. Such diaries can provide important clinical information as COPD patients with higher daily respiratory symptoms have been seen to be more susceptible to sudden onset of exacerbations (Aaron et al., 2012). Also, these diaries have the capability to pick up unreported exacerbations (identified by elevated respiratory symptoms) which can result in later severe exacerbations if left untreated (Donaldson et al., 2002; Calverley et al., 2005; Seemungal et al., 1998; Seemungal et al., 2000).

Despite the benefits of monitoring daily respiratory symptoms via daily diary cards, it is important to acknowledge there are limitations with the use of these tools. For example, daily diary cards do not have established thresholds or minimally clinically important differences (MCID) to counter for the heterogeneity of symptoms/exacerbations, making it challenging to establish clinically relevant changes in respiratory symptoms. The EXAcerbation of COPD Tool (EXACT[®]) has been developed and used in clinical studies to detect, monitor and assess the severity of exacerbations. The EXACT[®] is a daily quantitative patient reported measure assessing symptoms and functional capabilities (Jones et al., 2011). The overall score has been validated for detection of exacerbations and the severity of such events in clinical research by assessing the relationship among; EXACT[®] scores, medication use, SGRQ, stable-state FEV₁, GOLD stage and mMRC over time between acute and stable states (Leidy et al., 2011; Leidy & Murray, 2013). Within this measurement tool, there is a subscale (E-RS[™]) which focusses on

specific domains of respiratory symptoms; breathlessness, chest, and cough/sputum. This enables respiratory symptoms to be monitored prior to and during exacerbations to assess severity of and recovery from such events (Leidy & Murray, 2013). This tool is relatively new and thus far has been limited to the assessment of pharmacological treatments to prevent or treat COPD exacerbations (Beier et al., 2017; Papi et al., 2017; Wedzicha et al., 2014). However, there has been a lack of studies utilising this tool for the assessment of changes in symptoms in response to non-pharmacological interventions.

1.4.3 Exacerbation risk

Exacerbations have a hospital admission mortality rate of 10% (Steer, Gibson & Bourke, 2012), with up to 43% of patients dying within 1 year of admission (Connors et al., 1996).

Until recently, clinical measures to identify and predict the risk of future exacerbations had proved difficult. Relationships were also unable to be identified between clinical factors such as: FEV₁%, smoking history, body mass index (BMI), oxygen use, or mMRC dyspnoea score with patients who have recurrent exacerbations (Hurst et al., 2010; Moy et al., 2013). However, the large-scale Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) study outlined that the single best predictor of future exacerbations is a previous history of exacerbations (Hurst et al., 2010).

1.4.3.1 Frequent exacerbator phenotype

Exacerbations can be cluster events, whereby when a patient experiences an exacerbation, increased susceptibility to another event can be seen shortly after (Hurst et al., 2009). Patients who are more susceptible to, and experience, frequent exacerbations have been characterised as a distinct phenotype of COPD. A COPD phenotype is referred to as “a single or combination of disease attributes that

describe differences between individuals with COPD as they relate to clinically meaningful outcomes (symptoms, exacerbations, response to treatment, speed of progression of the disease or death)” (Han et al., 2010).

Frequent exacerbators are defined as COPD patients who have had 2 or more exacerbations in the last year (hospitalisations or treatment with antibiotics or corticosteroids), whereas infrequent exacerbators are COPD patients who have had none or 1 exacerbation in the last year (Hurst et al., 2010). The frequent exacerbator is considered to be a stable phenotype; however, the ECLIPSE study found that a significant proportion of COPD patients can see fluctuations in terms of exacerbation frequency (Donaldson et al., 2013).

The underlying mechanisms as to why a subset of patients experience recurrent exacerbations remains unclear. It has been proposed that patients who experience frequent exacerbations have an altered immune profile increasing susceptibility to recurrent infections (Wedzicha, Mackay & Singh, 2013). For example, airways of frequent exacerbators appear to be more inflamed with higher levels of IL-6 and IL-8 (Bhowmik et al., 2000). Furthermore, systemic biomarkers such as fibrinogen and CRP have also been seen to be elevated in frequent exacerbators (Donaldson et al., 2005a; Perera et al., 2007). This is further supported by evidence suggesting that patients with elevated levels of fibrinogen, CRP and leukocytes in a stable state have been suggested to be more at risk of having an exacerbation (Thomsen et al., 2013). The following mechanisms have been proposed to explain this underlying systemic inflammation in COPD: spill over of inflammatory mediators from the pulmonary compartment, inflammatory responses to tissue hypoxia, and a reaction induced by the pro-inflammatory bacterial product lipopolysaccharide (Wouters, 2005). Fibrinogen, CRP, and IL-6 are now recognised biomarkers of exacerbations playing prominent roles in the inflammatory processes in COPD; however, there is

still a need for clarity of the role of these biomarkers in the frequent exacerbator phenotype in relation to prediction of exacerbations (Chen, Leung & Sin, 2016).

Inflammation is heavily implicated in the frequent exacerbator phenotype and is regarded as a treatable trait of COPD, but currently the best treatment for inflammation is unclear (Agusti et al., 2016). In order to better understand the underlying biology, there have been calls for comparisons between frequent and infrequent exacerbators (Agusti & Vestbo, 2011). Much of the evidence so far has assessed changes in biological markers between patients in a stable state and when presenting with an exacerbation which has had limited use in informing treatments. The ECLIPSE study however, has assessed retrospective data and identified that frequent exacerbators of COPD present with elevated leukocyte counts, CRP and fibrinogen identifying potential surrogate markers of the treatable trait of inflammation (Hurst et al., 2010; Agusti et al., 2016). This has led to suggestions that measurements of inflammatory markers in the systemic compartment maybe more insightful moving forwards (Singh et al., 2014).

The frequent exacerbator phenotype is a recognised major phenotype across all severities of airflow limitation, however its prevalence does increase with COPD severity. The ECLIPSE study showed that frequent exacerbators were present in 22% of GOLD stage 2 COPD patients, 33% of stage 3, and 47% of stage 4 (Hurst et al., 2010), highlighting the importance of assessing exacerbation risk based on previous history in all COPD stages.

1.4.4 Combined COPD assessment

A revised assessment for COPD patients proposed in the GOLD, (2011), report and again updated in 2017 (GOLD, 2017), recognised the impact of exacerbations and has been a major step forward from using spirometry alone to diagnose and assess COPD to guide treatment. This combined assessment, through the categorising of

COPD patients through the GOLD stages (A, B, C, D), provides a more individualised treatment pathway by stratifying COPD patients based on assessment of symptoms and exacerbation risk following confirmed diagnosis via spirometry (Figure 1.2) (GOLD, 2017).

Exacerbation history	≥ 2 or ≥ 1 leading to hospital admission	C	D
	0 or 1 (not leading to hospital admission)	A	B
		mMRC 0-1 CAT < 10	mMRC ≥ 2 CAT ≥ 10
		Symptoms	

Figure 1.2. Adapted GOLD, (2017) combined ABCD assessment tool for COPD. GOLD stage A – COPD patients with low exacerbation risk and less symptoms. GOLD stage B – COPD patients with low exacerbation risk but more symptoms. GOLD stage C – COPD patients with high exacerbation risk with less symptoms. GOLD stage D – COPD patients with high exacerbation risk and more symptoms.

Further assessments (e.g. exercise tolerance) can also be undertaken to understand the capabilities of a patient and the functional impairment induced by COPD and/or comorbidities. An example of an exercise tolerance test is the incremental shuttle walk test (ISWT) which is a walking bleep test which gradually increases in speed until symptom limitation and is indicative of maximal exercise

capacity (Holland et al., 2014). However, the ISWT is not always suitable for patients with differing impairments meaning the 6-minute walk test (6MWT) can be performed instead. The 6MWT requires patients to walk as far as possible at their own pace in 6 minutes with rest periods permitted (Holland et al., 2014).

These assessment tools help to inform clinicians about treatment pathways on an individualised basis.

1.5 Treatment of COPD symptoms and exacerbations

COPD is a complex heterogeneous disease with many different options for treatment. The main therapeutic goals in the treatment of COPD are to reduce symptoms and the frequency of exacerbations (Vestbo et al., 2013).

1.5.1 Pharmacological therapy

Pharmacological therapies for the treatment of COPD fall under the two main categories of bronchodilators and anti-inflammatory treatments. Prescription of these pharmacological therapies depend on disease activity and severity. This is well characterised by the stepwise approach (Figure 1.3) (GOLD, 2019). The stepwise approach provides guidance on the implementation of pharmacological therapies as COPD progresses. For example, the use of bronchodilators in mild to moderate COPD to manage dyspnoea before introducing corticosteroids with more severe disease. Anti-inflammatory treatments such as corticosteroids are especially important for preventing and/or reducing the risk of exacerbations, hence often prescribed for frequent exacerbators (GOLD, 2019).



I: Mild	II: Moderate	III: Severe	IV: Very Severe
<ul style="list-style-type: none"> • $FEV_1/FVC < 0.70$ • $FEV_1 \geq 80\%$ predicted 	<ul style="list-style-type: none"> • $FEV_1/FVC < 0.70$ • $50\% \leq FEV_1 < 80\%$ predicted 	<ul style="list-style-type: none"> • $FEV_1/FVC < 0.70$ • $30\% \leq FEV_1 < 50\%$ predicted 	<ul style="list-style-type: none"> • $FEV_1/FVC < 0.70$ • $FEV_1 < 30\%$ predicted or $FEV_1 < 50\%$ predicted plus chronic respiratory failure
Active reduction of risk factor(s); influenza vaccination 			
Add short-acting bronchodilator (when needed) 			
Add regular treatment with one or more long-acting bronchodilators (when needed); add pulmonary rehabilitation			
Add inhaled glucocorticosteroids if repeated exacerbations			
Add long-term oxygen if chronic respiratory failure. Consider surgical treatments			

Figure 1.3. Stepwise approach to the treatment of COPD patients (GOLD, 2019).

1.5.1.1 Bronchodilators

Bronchodilators are a cornerstone of pharmacological therapy in COPD. Bronchodilators fall under two major classes, beta₂-adrenoceptor agonists (SABA and long-acting beta₂-adrenoceptor agonists (LABA)) and anti-muscarinic/anti-cholinergics (short-acting muscarinic antagonists (SAMA) and long-acting muscarinic antagonists (LAMA)) which are used as rescue medication and maintenance therapy in COPD (GOLD, 2019).

1.5.1.1.1 Beta₂-adrenoceptor agonists

Beta₂-adrenoceptor agonists, also known as beta-agonists, function via activation of the beta₂-adrenoceptors located on airway smooth muscle resulting in airway relaxation (Billington, Penn & Hall, 2017). SABA's play a key role in immediate relief of symptoms through accelerated bronchodilation and can be seen to reduce dynamic hyperinflation at rest and during exercise (O'Donnell et al., 2004; O'Donnell et al., 2006). SABA's are recommended to be prescribed upon diagnosis of COPD and can be used as a rapid 'reliever' across all disease severities (Figure 1.3). This inhaled medication can be seen to be effective within 3 min reaching peak bronchodilation after 2.5 hr to alleviate symptoms of dyspnoea (Ejiofor & Turner, 2013). SABA's can be seen to effectively act for between 4-6 hrs following intake (Vathenen et al., 1988).

LABA's function in a similar way to SABA's but provide a more prolonged response lasting 12 hrs or more (Cazzola et al., 2013). The prescription of a LABA is recommended for patients with moderate to very severe COPD (Figure 1.3). LABA's do not preclude any additional benefit from SABA's therapy as needed (Cazzola et al., 2013), and do not act as fast in terms of providing immediate relief of symptoms, but they have been seen to reduce exacerbations by 10-20% (GOLD, 2019; Wedzicha, Decramer & Seemungal, 2012).

The role bronchodilators play in prevention of exacerbations is believed to be attributed to a reduction in dynamic hyperinflation, a known mechanism underpinning increased breathlessness in COPD (Wedzicha, Decramer & Seemungal, 2012). Combining two LABA's can also induce significant improvements in lung function, but exacerbation risk is only marginally reduced (Wedzicha, Mackay & Singh, 2013). It is not believed that LABA's impact on the underlying causes of exacerbations, but plausibly increase the symptom threshold of COPD patients resulting in decreased exacerbations via reducing breathlessness (Vestbo & Lange, 2015) (Figure 1.4).

1.5.1.1.2 Anti-muscarinic/anti-cholinergics

Short-acting muscarinic antagonists are another form of bronchodilator which provide accelerated short-term bronchodilation via a different pathway to beta₂-agonists by blocking the activity of the muscarinic acetylcholine receptor, mainly M1 and M3-receptors, resulting in airway smooth muscle relaxation and reduced mucus secretion (Barnes, 1993). SAMA's can be utilised across all severities of COPD as a fast acting 'reliever' inhaler. LAMA's, such as Tiotropium, have been seen to have similar benefits to LABA's in terms of symptom relief, prolonged bronchodilation, and improvements in exercise tolerance but act via different mechanisms (Karner, Chong & Poole, 2012; Kesten et al., 2008). LAMA's should be prescribed in patients with moderate to very severe COPD (Figure 1.3). Reduced time to first exacerbation has also been seen when using tiotropium (LAMA) once-daily compared to salmeterol twice-daily (LABA) (Vogelmeier et al., 2011). LABA's are unlikely to have major anti-inflammatory effects (Powrie et al., 2007), whereas LAMA's could have a potentially beneficial effect by reducing mucus hypersecretion associated with exacerbations, although the data to support this mechanism are inconclusive (Vestbo & Lange, 2015).

A combination of these therapies can be implemented to optimise therapeutic response. It is becoming common place to prescribe LABA/LAMA combination therapy, with benefits such as short-term improvements in lung function and quality of life previously observed (Cazzola & Molimard, 2010; Martinez et al., 2017). There are several inhalers available which include both LABA's and LAMA's which have been shown to reduce the risk of exacerbations (Wedzicha et al., 2016). However, this effect has been questioned as a recent trial has found that the LABA/LAMA combination was no more effective than LAMA alone for reducing the risk of exacerbations (Calverley et al., 2018). This suggests that bronchodilators may need to be combined with anti-inflammatory therapies (e.g. corticosteroids) which are specifically designed to target inflammation to prove more effective in reducing exacerbations (Putman et al., 2016).

1.5.1.2 Anti-inflammatory agents

1.5.1.2.1 Corticosteroids

Inhaled corticosteroids (ICS) act to alleviate symptoms by reducing inflammation in the airways of COPD patients. Corticosteroids are targets for suppressing inflammation by activating transcription of anti-inflammatory and suppressing transcription of proinflammatory genes (Barnes, 2010). Mitogen kinase phosphatase-1 (MKP-1) and glucocorticoid-induced leucine zipper (GILZ) are both corticosteroid-inducible genes which are transcribed in response to corticosteroid treatment to prevent the release of pro-inflammatory mediators (Kelly et al., 2012; King et al., 2009; Newton & Holden, 2007). The main mechanism of MKP-1 is to inactivate pro-inflammatory mitogen-activated protein kinases (MAPK's) resulting in a reduced production of cytokines (Barnes, 2008). GILZ also targets key inflammatory transcription factors resulting in a suppression of inflammatory gene

expression (Berrebi et al., 2003; Eddleston et al., 2007; Godot et al., 2006; Mittelstadt & Ashwell, 2001).

Corticosteroids have largely been introduced as a treatment in COPD given the positive effects seen in the treatment of asthma. ICS treatment in COPD has been seen to be ineffective at arresting long-term FEV₁ decline (Burge et al., 2000; Pauwels et al., 1999; Vestbo et al., 1999; Wise et al., 2000), but has been seen to reduce exacerbation frequency (Spencer et al., 2004). Alone, corticosteroids have been shown to be effective in treating acute exacerbations and reducing exacerbation frequency by 25% (Burge et al., 2000; Calverley et al., 2007). The clinical benefits of corticosteroids have been established in COPD for reducing exacerbation events, but evidence does not always support a reduction in symptoms or of controlled inflammation (Barnes, 2000). This is believed to be as a result of decreased activity and expression of histone deacetylase 2 (HDAC2) in the inflammatory cells of COPD patients (Ito et al., 2005). This decreased HDAC2 activity is believed to be due to oxidative stress (Ito et al., 2005; Barnes, 2006). These proposals are considered to be a key factor in why COPD patients with neutrophilic inflammation are resistant to the effects of corticosteroids whereas COPD patients with eosinophilic inflammation, aligning with the inflammatory profile of asthma patients, respond better (Brightling et al., 2000; Chanez et al., 1997; Pizzichini et al., 1998; Pavord et al., 2016).

Systemic corticosteroids, as opposed to inhaled, are usually used to treat exacerbations and have been seen to induce improvements in lung function and dyspnoea whilst decreasing relapse rate in the treatment of exacerbations (Albert, Martin & Lewis; 1980; Niewoehner et al., 1999). However, the use of systemic corticosteroids is not associated with long-term benefits in stable COPD (Walters, Walters & Wood-Baker, 2005), and may increase the risk of mortality (Sin & Tu,

2001). The use of systemic corticosteroids has also been associated with side effects including weight gain and depression (Wood-Baker et al., 2005). Questions still arise over the effectiveness of both systemic and inhaled corticosteroid treatment as COPD patients have been suggested to develop a corticosteroid insensitivity as a result of chronic use, meaning that high doses fail to suppress inflammation (Lo Tam Loi et al., 2013).

Historically, corticosteroids have been targeted at patients who have frequent exacerbations despite treatment with LABA and/or LAMA (GOLD, 2019). The addition of ICS to the treatment regimen of LABA/LAMA has been recommended given the positive effect observed with a reduced risk of exacerbation (GOLD, 2019). However, there is an increasing presence of ICS withdrawal in COPD patients due to wide reports of pneumonia with long-term use (Fernandes et al., 2017), but the withdrawal of ICS has been seen to be associated with an increased rate of exacerbations of COPD (Magnussen et al., 2014; Watz et al., 2016; Wouters et al., 2005). Research has assessed the possibility of combination therapy (ICS & beta₂-agonists) to facilitate sensitivity to corticosteroids in COPD with suggestions of beneficial effects (Barnes, 2013; Boardman et al., 2014). Anti-inflammatory drugs (e.g. ICS) have different mechanisms in reducing the risk of exacerbations compared to LABA's. Anti-inflammatory drugs are assumed to modify the inflammatory response to airway irritants or bronchial infection accompanying an exacerbation (Vestbo & Lange, 2015). Anti-inflammatory drugs have a lesser effect on lung function compared to bronchodilators and have been suggested to only be effective in certain exacerbations depending on inflammatory profile (Vestbo & Lange, 2015). Furthermore, anti-inflammatory drugs target inflammation as a way of reducing symptomatic changes as opposed to bronchodilators which act to increase symptom thresholds for exacerbations (Figure 1.4).

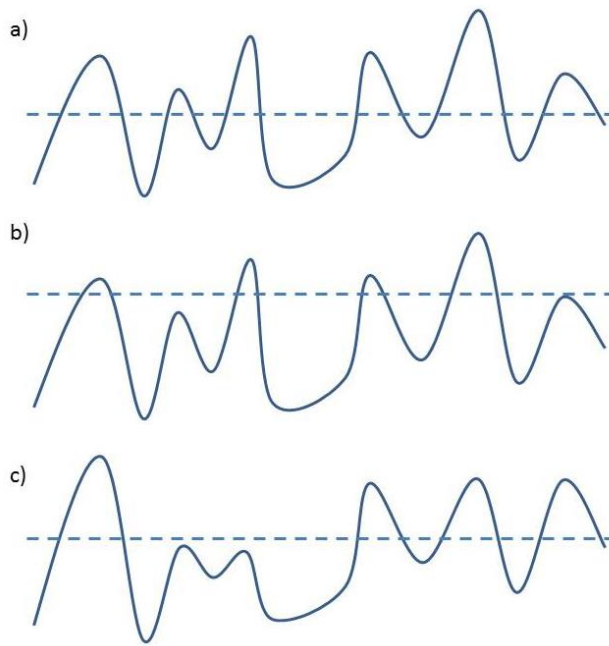


Figure 1.4. Symptomatic threshold for exacerbations and how pharmacological treatments can reduce exacerbation rate. a) Solid line dictates standard symptom variations over time with symptoms exceeding the exacerbation threshold as depicted by the dotted line. b) LABA's increase the symptom threshold line and exacerbation perception potentially reducing the amount of exacerbations experienced. c) Anti-inflammatory drugs do not alter the symptom threshold, but they: modulate inflammation, reduce symptoms, and reduce the amplitude of symptomatic changes, leading to fewer exacerbations (Vestbo & Lange, 2015).

1.5.1.2.2 PDE4 Inhibitors

Phosphodiesterase type 4 (PDE4) inhibitors (e.g. roflumilast) are a newer pharmacological therapy option for the treatment of COPD. This new therapy has been introduced following efforts to establish pharmacological regimens that specifically target inflammation and reduce exacerbations of COPD (Calverley et al., 2009). This is particularly relevant in the case of individuals who experience frequent exacerbations of COPD as there is a poor availability of treatments that specifically target inflammation in an effective manner (Han et al., 2014; Rabe & Watz, 2017). PDE inhibitors act by increasing levels of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) to relax the airway smooth muscle causing bronchodilation and inhibit production of

inflammatory cell activation (Bryson & Rodger, 1987; Cortijo et al., 1993; Holgate et al., 1987; Kotlikoff & Kamm, 1996; Schramm & Grunstein, 1992; Spina, 2003). Specifically, PDE4 inhibitors have been seen to reduce matrix metalloproteinase (MMP) activity, a protease capable of degrading extracellular matrix proteins, in the lung (Lagente et al., 2005). PDE4 inhibitors have also been shown to drive the resolution of neutrophilic inflammation (Sousa et al., 2010). As a result of these various anti-inflammatory mechanisms, PDE4 inhibitors have been seen to reduce the risk of exacerbations in COPD (Cowan, 2005; Rabe et al., 2005).

Ultimately, PDE4 inhibitors have found a place in the pharmacological management of COPD due to the lack of available anti-inflammatory treatments and the burden of recurrent exacerbations, but this therapy does come with severe side effects. These side effects include; nausea, vomiting and increased gastric acid secretion (Dyke & Montana, 2002), and use of this therapy should be undertaken with caution (GOLD, 2019).

1.5.1.3 Pharmacological issues

Large sums of money are spent on pharmacological therapies to treat COPD (World Health Statistics, 2011), however, the benefits of these therapies on exacerbations, hospitalisations or mortality are modest at best and/or at the expense of the incidence of adverse effects (Sin, 2014). The pharmacological options currently appear to be insufficient in suppressing inflammation, which is considered to be a treatable trait in COPD (Agusti et al., 2016). With this in mind, the importance of non-pharmacological treatment options are on the rise. Vestbo et al. (2013) have emphasised the multi-component nature of non-pharmacological interventions suggesting that interventions such as; smoking cessation, pulmonary rehabilitation and physical activity could play an important role in the management of symptoms and exacerbations.

1.5.2 Non-Pharmacological therapy

1.5.2.1 Smoking cessation

Despite a depth of pharmacological and non-pharmacological treatment options, smoking cessation is still considered the intervention with greatest potential to influence the management of COPD patients and is strongly recommended by health services and government bodies (Hopkinson & Polkey, 2010). However, damage as a result of chronic smoking has already occurred and smoking cessation cannot reverse these effects, so it is important to also seek supporting treatments to limit the progression of COPD (Hopkinson & Polkey, 2010).

Smoking is believed to be a major contributing factor to COPD exacerbations, with a high prevalence of COPD exacerbations in patients who continue to smoke (Badaran et al., 2012). Smokers with respiratory symptoms may also be more susceptible to the effects of smoking than those who do not present with symptoms (Kohansal et al., 2009). However, smoking cessation is believed to improve respiratory symptoms and prevent excessive lung function decline in smokers with COPD as well as among smokers without chronic symptoms (Willemse et al., 2004).

1.5.2.2 Flu vaccination

Another cost-effective non-pharmacological treatment in COPD is influenza vaccination (Lall et al., 2016). Influenza represents a key cause of COPD exacerbations which have a significant burden on healthcare utilisation (Seemungal et al., 2001). Influenza vaccinations have been seen to reduce lower respiratory tract infections requiring hospitalisation (Wongsurakiat et al., 2004), and death in COPD patients (Nichol et al., 1994; Poole et al., 2006), meaning they are strongly recommended for patients with COPD (Bekkat-Berkani et al., 2017).

1.5.2.3 Education

Patient education has been suggested to be an independently effective treatment pathway for COPD, despite the inability of education to independently alter lung function or exercise capacity (Celli, 1995). Education is intended to support long-term behaviour change and has been seen to play a role in; improving skills, instilling confidence in self-management options, improving ability to handle disease complications, enhancing smoking cessation, and health status if used effectively (Celli, 1995; GOLD, 2019).

1.5.2.4 Physical activity

Reduction in physical activity levels during the course of COPD increases the risk of comorbidity development and disease progression (Garcia-Aymerich et al., 2007). COPD patients are commonly seen to be less physically active than healthy individuals with further decline in physical activity levels and larger portions of the day being spent in a sedentary state as COPD severity worsens (Pitta et al., 2005; Watz et al., 2009). As a result, COPD patients who have lower physical activity levels are likely to have a poorer survival rate and increased risk of hospitalisation compared to COPD patients who are physically active (e.g. 2 or more hours of physical activity per week) (Garcia-Aymerich et al., 2006).

In COPD, a vicious cycle involving breathlessness, inactivity and reduced exercise capacity resulting in poorer quality of life has been proposed (Troosters et al., 2013). This vicious cycle illustrates the negative effects of continued inactivity in COPD, but this cycle can be reversed with an increase in physical activity. COPD patients who maintain increased physical activity levels are associated with improved lung function or slower lung function decline, improved functional status, and when physical activity is undertaken at a low intensity can experience a lower frequency of exacerbations and hospitalisations (Donaire-Gonzalez et al., 2015;

Garcia-Aymerich et al., 2007; Garcia-Aymerich et al., 2009). Despite this, there is still a clear lack of understanding of the mechanisms underpinning these changes.

The norm for promoting physical activity in COPD is to deliver structured physical activity in the form of an exercise programme to help with disease management (Troosters et al, 2013).

1.5.2.5 Pulmonary rehabilitation

Exercise training, most often as part of pulmonary rehabilitation, is considered a cornerstone treatment in the management of COPD. Pulmonary rehabilitation has been defined as “a comprehensive intervention based on a thorough patient assessment followed by patient-tailored therapies that include, but are not limited to, exercise training, education, and behaviour change, designed to improve the physical and psychological condition of people with chronic respiratory disease and to promote the long-term adherence to health-enhancing behaviours” (Spruit et al., 2013).

The NICE, (2018) guidelines recommend that patients with stable COPD who are functionally impaired due to breathlessness should be referred for ‘conventional’ pulmonary rehabilitation. NICE guidance also recommends that patients admitted to hospital for an acute exacerbation of COPD should be referred for and commence ‘early’ pulmonary rehabilitation within 4 weeks of discharge (NICE, 2018). This quality standard has been released as NICE suggest the commencement of early pulmonary rehabilitation post-discharge for acute exacerbation of COPD reduces the short-term risk of hospital readmission whilst improving quality of life and exercise capacity (NICE, 2018).

UK recommendations for conventional and early pulmonary rehabilitation programmes include a programme of 6-12 weeks, with 2 or 3 sessions per week, to

achieve noticeable improvements in quality of life and exercise capacity (Bolton et al., 2013). Pulmonary rehabilitation can take place in a variety of settings including; community settings, hospital, and patients' homes with clinical benefits demonstrated in all settings if delivered in line with national guidelines (Bolton et al., 2013). Pulmonary rehabilitation has been shown to have moderate to large clinically significant improvements on the management of dyspnoea, increasing exercise capacity, and improving quality of life for patients with COPD (McCarthy et al., 2015; Nici et al., 2006). Given the in-depth evidence around the benefits of pulmonary rehabilitation on outcomes such as exercise capacity and quality of life, McCarthy et al. (2015) concluded that there was no need for further randomised controlled trials comparing the effect of pulmonary rehabilitation to usual care in COPD on these outcomes (McCarthy et al., 2015). However, this was concluded based on the average COPD patient, whereby due to the inherent heterogeneity in COPD, some patients respond better than others, underlying the importance of looking into comorbidity and phenotype subsets and how these patients respond to rehabilitation (Spruit et al., 2015a).

The aforementioned study by Spruit et al. (2015a) categorised patients as 'very good responders', 'good responders', 'moderate responders', and 'poor responders'. Key factors underpinning 'very good responders' included COPD patients with; higher symptoms of dyspnoea, higher number of hospitalisations in the previous 12 months, poorer exercise performance, and more symptoms of anxiety and depression. This paper demonstrated the importance of considering subgroups of COPD patients in the context of pulmonary rehabilitation. However, a subsequent study has followed on from this to suggest that pulmonary rehabilitation stood to benefit all COPD patients regardless of the presence, number, or nature of comorbidities (Kallianos et al., 2016). Interestingly, many of the listed

characteristics of 'very good responders' align with characteristics of the frequent exacerbator phenotype (Le Rouzic et al., 2018), yet there is still a poor understanding of where pulmonary rehabilitation sits in the treatment of this phenotype, and other clinical phenotypes, especially given the poor efficacy of pharmacological options.

Despite the benefits of using pulmonary rehabilitation as a treatment, the funding provided is far less than that provided to lesser effective treatments (Griffiths et al., 2001; Hak et al., 1998; Hoogendoorn et al., 2010; Mayers et al., 2007; Oba, 2007). Pulmonary rehabilitation has routinely not been considered to impact on outcomes such as reduced health care use and disease progression which may, in part, explain this shortfall in funding from healthcare services burdened by COPD exacerbations. Another major issue identified with pulmonary rehabilitation is the low rates of referral and poor patient uptake (Brooks et al., 2007; Garvey, Fullwood, & Rigler, 2013; Hayton et al., 2013; Jones et al., 2014b; Jones et al., 2017; Keating, Lee, & Holland, 2011; Marciniuk et al., 2010). Numerous qualitative studies have identified common patient reported barriers linked to this poor uptake including a lack of perceived benefit or a fear that exercise (pulmonary rehabilitation) may exacerbate symptoms (Fischer et al., 2007; Harris, Hayter & Allender, 2008; Taylor et al., 2007; Young et al., 1999).

Even when enrolled, completion of pulmonary rehabilitation is another significant issue for providers with attrition rates as high as 60% (Boutou et al., 2014; Hayton et al., 2013; Hogg et al., 2012). One factor that often has been proposed to be related to poor adherence is incidence of exacerbations during pulmonary rehabilitation programmes (Hayton et al., 2013). Up to one third of dropouts from pulmonary rehabilitation have been attributed to acute exacerbations of COPD (Steele et al., 2010). However, a recent study has suggested that this is limited to

severe exacerbations whereas mild-to-moderate exacerbations do not affect dropout or response to pulmonary rehabilitation (Braeken et al., 2017). Such evidence is important to consider when referring COPD patients with a history of exacerbations to pulmonary rehabilitation but it remains unclear to what extent the frequent exacerbator phenotype impacts on programme compliance and outcomes across all pulmonary rehabilitation settings (e.g. home, community and hospital).

1.6 Exercise in the management of COPD exacerbations

1.6.1 Pulmonary rehabilitation & exacerbations

More recently, interest has grown in assessing whether pulmonary rehabilitation has impact beyond exercise capacity and health status (McCarthy et al., 2015). There has been increasing numbers of reports of pulmonary rehabilitation reducing the risk of health-care use (Griffiths et al., 2000; Moore et al., 2016; van Ranst et al., 2014). A recent systematic review by Moore et al. (2016) found that pulmonary rehabilitation groups had lower overall rates of hospital admissions compared to those in control groups in randomised controlled trials. Five of the studies included in this review were pooled and found that pulmonary rehabilitation reduced hospital admissions in the 12 months post-rehabilitation compared to the 12 months prior to rehabilitation (Moore et al., 2016). However, this review also considered pooled data from observational cohort studies where findings were in contrast with randomised trials and did not support a beneficial effect of pulmonary rehabilitation on hospitalisations over control groups (Moore et al., 2016). The same authors also performed an historical cohort study using electronic health records and suggested that patients referred for pulmonary rehabilitation (but did not necessarily complete) did not have fewer GP visits and hospitalisations for acute exacerbations of COPD in the year following pulmonary rehabilitation compared to those not referred to pulmonary rehabilitation (Moore et al., 2017). However, 'real-world' data in the form

of the UK National COPD Pulmonary Rehabilitation Audit has highlighted a reduction in the risk of hospitalisation and length of stay following completion of pulmonary rehabilitation (Steiner et al., 2017a). These findings highlight the lack of clarity and interest in assessing health-care use outcomes in response to exercise interventions especially following pulmonary rehabilitation to identify if the duration of benefits from a pulmonary rehabilitation programme alone can be prolonged, or rather, enhanced during the post-rehabilitation period (Moore et al., 2016).

Early pulmonary rehabilitation (1-month post-hospital discharge for acute exacerbation) has also been shown in clinical trials to reduce hospital readmissions in COPD patients (Puhan et al., 2011). A recent update in the synthesis of evidence in this area has downgraded the quality of the evidence due to inconsistencies in the estimates of effect across randomised controlled trials to date (Puhan et al., 2016). However, the authors felt that the extensiveness of the programmes delivered, i.e. the amount of exercise undertaken, was key in explaining the variance in intervention effect suggesting more research is required in this area (Puhan et al., 2016).

The benefits outlined have seen pulmonary rehabilitation labelled as one of the most cost-effective methods of treating COPD (California Pulmonary Rehabilitation Collaborative Group, 2004; Griffiths et al., 2000; Griffiths et al., 2001; Puhan et al., 2011; Raskin et al., 2006; Zoumot, Jordan, & Hopkinson, 2014). Therefore, any patients who have the potential to benefit from pulmonary rehabilitation are strongly recommended to be offered this therapy (Rochester et al., 2015). It is also important to note that pulmonary rehabilitation was not designed or intended to impact health care use but the mounting evidence linking the positive interaction cannot be ignored.

Isolating the component of pulmonary rehabilitation largely responsible for this reduction in exacerbations is difficult. It is unclear whether the observed effect is due to the exercise alone, or whether other factors (e.g. education) are having a sole or additive effect. For example, Evans & Steiner (2018) proposed that the role pulmonary rehabilitation plays in increasing disease knowledge may contribute to this reduction in exacerbations. Further investigation of the efficacy and mechanisms of the impact of exercise interventions on exacerbations would help to answer this.

1.6.2 Exercise maintenance and exacerbations

Given the numerous benefits identified with pulmonary rehabilitation, there are still issues surrounding long-term sustainability of improvements gained from pulmonary rehabilitation, with patients returning to baseline levels 12 months following rehabilitation (Mador, Patel & Nadler, 2011; McCarthy et al., 2015). Therefore, there is interest in exercise programmes which can maintain the initial benefits of pulmonary rehabilitation (Alison et al., 2017; Beauchamp et al., 2013).

Supervised maintenance exercise programmes after pulmonary rehabilitation in COPD appear to be more effective in preserving the improvements in exercise capacity up to 6 months but show no effects with respect to health-related quality of life post-rehabilitation (Beauchamp et al., 2013; Busby, Reese & Simon, 2014). Exacerbations and hospital admissions are key events in the management of COPD but the effects of exercise, particularly supervised maintenance programmes following pulmonary rehabilitation, on these outcomes have received little attention. As maintenance programmes are usually exercise only programmes, it provides a model by which to further assess the effects of exercise alone on exacerbations and other health care use outcomes.

Therefore, a systematic review was undertaken (Jenkins et al., 2018), using a pre-registered protocol (Appendix A) to collate and synthesise all of the available evidence from randomised controlled trials in order to estimate the size of the effect of supervised maintenance exercise programmes compared to usual care following pulmonary rehabilitation on health-care use (Table 1.1).

Table 1.1. Characteristics of included studies

Study (Country)	Sample size, Gender, Age	FEV ₁ % predicted (spirometry), Smoking history	Inclusion/ Exclusion criteria	Study aim, Design, Unit of allocation	Pulmonary rehabilitation programme (setting, components, duration, frequency)	Maintenance programme (setting, components, duration, frequency)	Primary outcome (1) Other outcomes and follow up (2)
Ries <i>et al.</i> , 2003 (USA)	164 participants Int: n = 83 Con: n = 81 Males: n = 89 Females: n = 75 Age, mean \pm SD All: 67 \pm 8	FEV ₁ %pred, mean All: 45% No data available for smoking status	Inclusion: Clinical diagnosis of chronic lung disease; Chronic symptoms and perceived disability from disease; Stable state; No other significant medical or psychiatric conditions that would interfere with programme participation; Commitment to abstain from smoking.	Assess a telephone-based maintenance intervention for retaining benefits following pulmonary rehabilitation RCT, cluster	Exercise and education combined with psychosocial support. Twelve, 2 h sessions over 8 weeks.	Weekly semi-structured phone calls and monthly supervised reinforcement sessions (1.5 h supervised exercise, 1.0 h topic review, 0.5 h social time) for 12 months.	(1) Pulmonary function, exercise tolerance, dyspnoea, depression. (2) QoL, health status, health-care use.
Brooks <i>et al.</i> , 2002 (Canada)	85 participants Int: n = 37 Con: n = 48 Males: n = 50 Females: n = 35 Age, mean \pm SD Int: 68 \pm 1 Con: 68 \pm 1	FEV ₁ %pred, mean \pm SD Int: 32 \pm 2 Con: 32 \pm 2 All non-smokers for at least 6 months	Inclusion: Severe stable COPD (FEV ₁ <40% predicted, FEV ₁ /FVC <0.70); Completion of inpatient or outpatient rehabilitation; Non-smoker for a minimum of 6 months; Aged 49-85 years. Exclusion: Coexisting conditions that might limit exercise tolerance or cognitive functioning; Non-compliance with respiratory rehabilitation; Mechanical ventilatory support for any part of the day; Inability to communicate in English; Living too far away to participate.	Compare the effects of two post-rehabilitation programmes on functional exercise capacity and health-related QoL in patients with COPD. RCT, individual	Exercises – Breathing, treadmill or cycle exercises, interval and upper extremity training, leisure walking. Patient education and psychosocial support included (relaxation and occupational therapy). Inpatient – 5 times a week for 6 weeks. Outpatient – 3 times a week at the centre and at home for 8 weeks.	Monthly 2 h group sessions supervised by a physical therapist for 12 months. First hour for discussion around home exercise programme, second hour for performing components of the home exercise programme under supervision. Phone calls made between visits with standardised questions regarding	(1) 6MWT, CRQ. (2) Medical outcomes survey: short-form 36, SGRQ, subject compliance, pulmonary function.

						adherence to home exercises.	
Spencer <i>et al.</i> , 2010 (Australia)	48 participants Int: n = 24 Con: n = 24 Males: n = 22 Females: n = 26 Age, mean \pm SD Int: 65 \pm 8 Con: 67 \pm 7	FEV ₁ %pred, mean \pm SD Int: 57 \pm 21% Con: 60 \pm 16% Current smokers Int: n = 6 Con: n = 5	Inclusion: COPD diagnosis; Completed 8-week pulmonary rehabilitation programme; FEV ₁ /FVC < 70% and FEV ₁ < 80% predicted. Exclusion: Exacerbation in previous month; Supplemental oxygen; Comorbidities that would prevent performing exercises; Clinic patients (pulmonary rehabilitation).	Determine if weekly-supervised exercise following pulmonary rehabilitation would maintain functional exercise capacity and QoL. RCT, individual	Exercises – 20 min walking, 20 min cycling, 10 min arm cycling, upper and lower limb strength training. 8 weeks in a pulmonary rehabilitation gym.	Pulmonary rehabilitation gym for 12 months. Supervised exercise 1 day per week with unsupervised exercise 4 days per week. Exercises prescribed in line with exercise undertaken during pulmonary rehabilitation.	(1) 6MWT, SGRQ. (2) Lung function tests, ISWT + ESWT, HADS, hospital admissions, length of stay and exacerbations.
Ringbaek <i>et al.</i> , 2010 (Denmark)	96 participants Int: n = 55 Con: n = 41 Males: n = 31 Females: n = 63 Age, mean \pm SD Int: 67 \pm 1 Con: 69 \pm 9	FEV ₁ %pred, mean \pm SD Int: 36 \pm 14% Con: 37 \pm 16% Current smokers Int: n = 13 Con: n = 7	Inclusion: Stable COPD (FEV ₁ < 80%, FEV ₁ /FVC < 70%); Motivation for pulmonary rehabilitation; Completion of 7 weeks of pulmonary rehabilitation. Exclusion: Musculoskeletal, cardiac or cognitive problems.	Examine whether maintenance training improved long-term effect of pulmonary rehabilitation. RCT, individual	Supervised walking and cycling both at 85% of predicted VO _{2 peak} + unsupervised exercise at home. Twice a week for 7 weeks with supplementary education once a week.	Weekly supervised exercise for first 6 months, every second week for the next 6 months and no supervised exercise for the last 6 months. Unsupervised exercise at home encouraged.	(1) ESWT, SGRQ. (2) Hospitalisation (time to first admission, admission rates, days in hospital), exercise adherence, attendance at evaluation visits.
Wilson <i>et al.</i> , 2015 and Burns <i>et al.</i> , 2016 (United Kingdom)	148 participants Int: n = 73 Con: n = 75 Males: n = 91 Females: n = 57 Age, mean \pm SD Int: 67 \pm 15 Con: 69 \pm 9	FEV ₁ %pred, mean \pm SD All: 41 \pm 16% >20 pack-year smoking history No data available on current smoking status	Inclusion: Over 35 years of age; COPD diagnosis (FEV ₁ < 80%); >20 pack-year smoking history; Completed at least 60% of pulmonary rehabilitation sessions. Exclusion: Cardiac or pulmonary disease (other than COPD); Myocardial infarction within 6 months or unstable angina; Respiratory infection within last 4 weeks; Uncontrolled or severe comorbidities; Cognitive complications.	Evaluate long-term effect of maintenance exercise on health-related QoL. Assess the cost-effectiveness of maintenance exercise following pulmonary rehabilitation on	Exercises - Walking, cycling, sit to stand, step-ups, arm exercises with dumbbells. High intensity (85% of maximum capacity). Once a week for 8 weeks (1 hour for exercise + 1 hour for education). Endurance exercise everyday	Individually tailored strength + endurance exercises including walking, cycling, sit-to-stand, step-ups and arm exercises with dumbbells. One 2 h (1 h exercise + 1 h education) session every 3 months for 12 months. Same	(1) CRQ (dyspnoea). (2) CRQ (other domains), ESWT, BMI, Body fat, HADS, EQ5D.

				health-related QoL. RCT, individual	+ strength exercise two more times a week at home.	group of patients from original pulmonary rehabilitation. Home exercise programme review.	
Roman <i>et al.</i> , 2013 (Spain)	71 participants Con: n = 23 RHB: n = 22 RHBM: n = 26 Males: n = 58 Females: n = 13 Age, mean (95% CI) RHBM: 65 (62 - 68) RHB: 64 (60– 68) Con: 63 (60 - 66)	FEV ₁ %pred, mean (95% CI) Con: 60 (56 - 64) RHB: 60 (55 - 65) RHBM: 61 (56 - 66) Current smokers Con: n = 8 RHB: n = 7 RHBM: n = 9	Inclusion: 35 – 74 years old; Moderate COPD diagnosis; Smokers or non-smokers. Exclusion: Musculoskeletal conditions affecting ability to exercise; Terminal illness/other severe disease.	Use maintenance post-pulmonary rehabilitation to improve QoL in COPD RCT, individual	Exercises - Low intensity peripheral muscle training. Abdominal, upper and lower limb exercises, shoulder and full arm circling, weight-lifting and other exercises. Each exercise repeated 8 -10 times over 45 min. Three 1 h sessions a week for 12 weeks with 15 min of respiratory physiotherapy every session and 45 min of education during weeks 1, 6 + 12.	Low intensity peripheral muscle training. One session a week for 9 months.	(1) CRQ. (2) Pulmonary function, 6MWT, hospital admissions, GP visits, exacerbations.

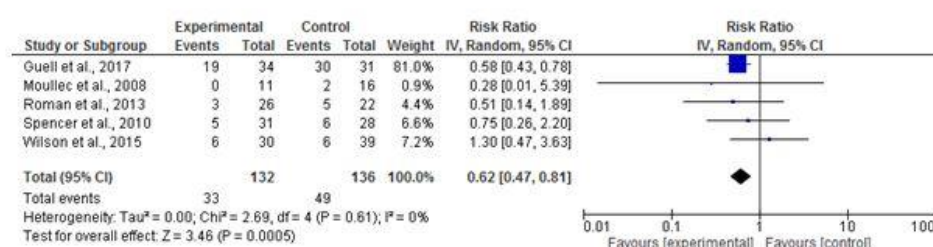
Moullec <i>et al.</i> , 2008, 2010 (France)	40 participants Int: n = 14 Con: n = 26 Males: n = 31 Females: n = 9 Age, mean \pm SD Int: 63 \pm 7 Con: 60 \pm 10	FEV ₁ %pred, mean \pm SD Int: 53 \pm 16 Con: 47 \pm 19 No data available on smoking status	Inclusion: FEV ₁ /FVC <0.7, FEV ₁ 30-79% predicted; No indication for home oxygen therapy; Stable state for the previous 2 months; No change in medication and symptoms for the previous 4 weeks; >40 years of age; No previous pulmonary rehabilitation experience. Exclusion: Medical or psychiatric disturbances that would hinder programme participation; Diagnosis of asthma; Congestive left heart failure; Terminal disease.	Determine changes in the emotional and functional dimensions of QoL in COPD one year after a pulmonary rehabilitation programme with or without a follow-up intervention. Quasi-RCT, individual	Twenty inpatient sessions over 4-weeks.	Community gymnasium. Individualised strength, interval, breathing, and endurance training with nature walking at ventilatory threshold. 96 sessions across 12 months. Exercise training (3.5 h per week; 72 sessions); health education (2 h per month; 12 sessions); and psychosocial support (with discussion group 1 h per month; 12 sessions).	(1) 6MWT, QoL (SGRQ and WHOQoL-Brief). (2) Six-item questionnaire with a VAS, maximal exercise capacity, physical activity, health-care utilisation, attendance, pulmonary function.
Guell <i>et al.</i> , 2017 (Spain)	138 participants Int: n = 68 Con: n = 70 Males: n = 123 Females: n = 15 Age, mean \pm SD Int: 64 \pm 9 Con: 64 \pm 8	FEV ₁ %pred, mean \pm SD Int: 34 \pm 11 Con: 34 \pm 9 No data available on smoking status	Inclusion: COPD diagnosis (grade II-IV severity); Clinically stable during previous 4 weeks; 18-75 years old; Ex-smokers or with intention to quit; BODE index value between 3-10. Exclusion: Bronchodilator response (FEV ₁ increment >15% of the baseline value after 200mcg of inhaled bronchodilator); Other respiratory diagnosis; Severe coronary artery disease; Orthopaedic diseases limiting mobility; Life expectancy lower than 2 years; Inability to co-operate.	Assess the efficacy of a supervised maintenance programme after pulmonary rehabilitation on improving symptoms, exercise capacity and health-related QoL compared with just pulmonary rehabilitation on its own. RCT, individual	Three hospital-based 2 h sessions a week for 8 weeks. Supplemented with 4 education sessions and chest physiotherapy. 30 min weight-lifting (0.5 kg in each hand, increased by 1 kg a week until peak tolerance), 30 min leg cycling (start at 50% maximum load achieved during initial exercise test, load increased by 10 Watts if heart rate	Supervised exercise on alternate weeks at hospital for 36 months. Unsupervised home exercise programme (3 days a week) similar to hospital programme (15 min chest physiotherapy, 30 min arm training, 30 min leg training). Supplemented by structured phone calls from physiotherapists every 15 days.	(1) BODE index. (2) 6MWT, Health-related QoL and CRQ.

					and oxygen saturation are stable and exercise is tolerated).	Exercise similar to pulmonary rehabilitation. Exercises, if well tolerated, were progressed at hospital visits.	
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Con = control group, Int = intervention group, RHB = pulmonary rehabilitation with no maintenance, RHBM = pulmonary rehabilitation with maintenance, RCT = randomised controlled trial, FEV₁ %pred = forced expiratory volume in 1 second % of predicted, FVC = forced vital capacity, QoL = quality of life, 6MWT = 6 minute walk test, SGRQ = St. Georges respiratory questionnaire, ISWT = incremental shuttle walk test, ESWT = endurance shuttle walk test, HADS = hospital anxiety and depression scale, CRQ = chronic respiratory disease questionnaire, BMI = body mass index, EQ5D = euro quality of life five dimensions questionnaire, WHOQoL = world health organisation quality of life brief questionnaire, VAS = visual analogue scale, BODE = body mass index, airflow obstruction, dyspnoea, and exercise index.

Data synthesis of five trials (Guell et al., 2017; Moullec et al., 2008 & 2010; Roman et al., 2013; Spencer, Alison & McKeough, 2010; Wilson et al., 2015) suggests that, on average, supervised maintenance exercise following pulmonary rehabilitation compared to pulmonary rehabilitation alone significantly reduces the risk of experiencing at least one respiratory-cause hospital admission by 38% (Figure 1.5A). Synthesized data from three trials (Guell et al., 2017; Spencer, Alison & McKeough, 2010; Wilson et al., 2015), suggest that, on average, supervised maintenance exercise may also influence multiple admissions by reducing the rate of respiratory-cause admissions by 28% (Figure 1.5B).

A



B

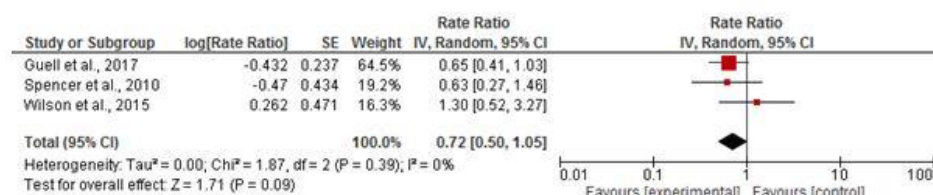


Figure 1.5. Trial-level data, effect estimates and forest plot of comparison for the overall risk (of experiencing at least one event) (A) and incidence rates (B) of respiratory-cause hospitalisation.

Whilst pooling of studies for other primary measures in this review (relative risk-reduction in exacerbations requiring treatment with antibiotics and/or systemic corticosteroids and all-cause mortality of 21% and 43% respectively) did not translate to statistically significant changes, the point estimates of effect do surpass

proposed thresholds of clinical significance (Jones et al., 2014a; Wedzicha et al., 2017) (Figure 1.6 & Figure 1.7).

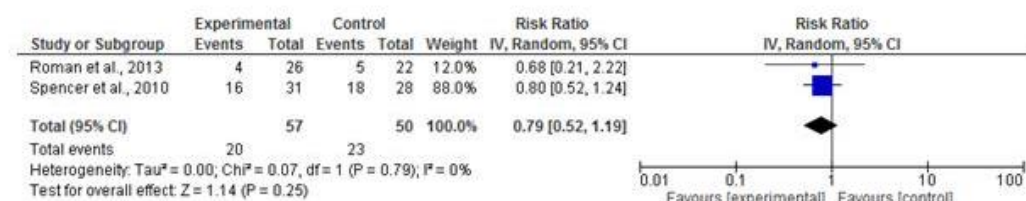


Figure 1.6. Trial-level data, effect estimates and forest plot of comparison for the overall risk of experiencing at least one exacerbation requiring treatment with medication.

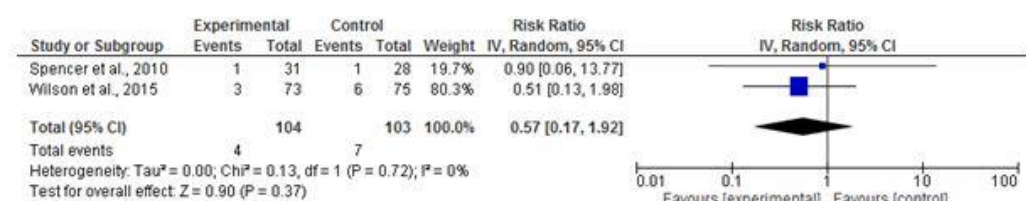


Figure 1.7. Trial-level data, effect estimates and forest plot of comparison for the risk of mortality.

Similarities, with regards to the benefits of exercise on COPD hospitalisation, can be seen with Moore et al. (2016) where data from randomised controlled trials on health-care use following pulmonary rehabilitation alone compared to no treatment were synthesised. However, the Moore et al. (2016) review did not focus on interventions aiming to maintain exercise regimens following pulmonary rehabilitation, but instead evaluated the short- and long-term benefits of initial pulmonary rehabilitation programmes on exacerbations compared to no treatment. It was concluded that the delivery of pulmonary rehabilitation to stable COPD patients or patients following acute exacerbations results in reduced rates of hospitalisations compared with usual care. The findings presented above suggest that continuing maintenance exercise in a supervised manner following pulmonary rehabilitation may further enhance the benefit on hospitalisations, supporting proposals for an independent effect of exercise on health-care use in COPD. Such evidence of the efficacy of exercise alone on incidence of exacerbations further

strengthens the potential value of non-pharmacological therapies in the management of COPD patients with a history of frequent exacerbations. It also suggests that the underlying mechanism of the impact of such interventions (e.g. pulmonary rehabilitation) on exacerbations are linked, in part, to effects beyond improvements in disease knowledge/management (Evans & Steiner, 2018), and may in fact be immune-regulatory or anti-inflammatory effects of exercise (Jenkins, Holden & Jones, 2018). To investigate this further, it would be important to compare inflammatory responses to pulmonary rehabilitation and/or physical activity according to COPD exacerbation phenotypes to determine any potential anti-inflammatory mechanisms with exercise.

1.6.3 Exercise & mechanisms of exacerbation & symptom management

Despite reports of the benefits of exercise-based programmes (pulmonary rehabilitation (Moore et al., 2016), and supervised maintenance exercise following rehabilitation (Jenkins et al., 2018)), on health-care use outcomes such as COPD exacerbations, there is still a paucity of evidence examining the underpinning mechanisms (Jenkins, Holden & Jones, 2018). However, in healthy populations, there is a body of research demonstrating the effects of exercise on relevant mechanistic pathways seen in COPD exacerbations (inflammation, respiratory symptoms/infections and immunity). These will be explored in this section and subsequently discussed to what extent they have been transferred to COPD populations yet.

1.7 Exercise as an anti-inflammatory intervention in healthy

It is well established that exercise induces transient changes on immune and inflammatory responses that are dependent on exercise intensity, duration and frequency (Woods et al., 2012). In healthy populations, moderate exercise intensity and frequency has been suggested to enhance immunity with prolonged strenuous

exercise suggested to decrease immunity (Walsh et al., 2011). The immune response to exercise involves transient changes in several markers of host defence and inflammation depending on whether assessments are based on chronic or acute effects of exercise (Kruger & Mooren, 2007; McCarthy & Dale, 1988).

1.7.1 Inflammatory cells

1.7.1.1 Chronic responses

Leukocytes and neutrophils play a key role in the innate immune response as a first line of defence against invading pathogens (Bouman et al., 2004; Malm, Lenkei & Sjodin, 1999; Nieman, 2001). Regular exercise training has been suggested to have little impact on blood total leukocyte and neutrophil counts in healthy populations (Gleeson & Bishop, 2005). However, there is little understanding on the role exercise training may play in neutrophil function (Walsh et al., 2011). Walking interventions, in the form of ~30-60 min 2 days a week for 12 weeks, has been observed to provide immunological benefits and attenuate oxidative stress when compared to a control group (Takahashi et al., 2013). In terms of neutrophil activation, this study reported increased expression of the adhesion marker CD62L, which is commonly shed (i.e. reduced) upon neutrophil activation, with a concomitant decrease in the activation marker CD66b, both findings indicative of a protective effect against inflammation (Takahashi et al., 2013). This proposes that exercise training in a healthy population, where leukocyte and neutrophil counts are at normal levels, may play more of a role in reducing basal neutrophil activation marker expression as opposed to decreasing inflammatory cell numbers. However, the effects of exercise training in populations characterised by inflammation (i.e. increased leukocyte and neutrophil counts) are unclear.

1.7.1.2 Acute responses

It is widely accepted that an acute bout of exercise in a healthy population induces an inflammatory response as characterised by an increase in leukocyte and neutrophil counts (Petersen & Pedersen, 2005; Walsh et al., 2011). The initial response of increased activation and numbers in circulation is an evolutionary response to physical stress, and the preparing of the body for immune responses to prevent injury or infection (Pedersen & Hoffman-Goetz, 2000). A second delayed increase in neutrophil count is also seen in the hours following exercise which is related to the intensity and duration of the acute bout of exercise (Peake, 2002; Robson et al., 1999). This delayed neutrophilia following more strenuous exercise is a temporary immune response with circulating neutrophil levels returning to baseline levels within 24 hours of exercise cessation depending on the type of exercise undertaken (Mooren et al., 2012).

Mature neutrophils are mobilised from the marginated pool during exercise (Walsh et al., 2011), with immature neutrophils sourced from the bone marrow if acute exercise is strenuous (Risoy et al., 2003). Not only does exercise alter neutrophil mobilisation, but exercise can also be seen to alter the function of these inflammatory cells. An acute bout of exercise has been suggested to result in an enhanced neutrophil activation as characterised by increases in neutrophil activation markers (CD11b & CD66b) (Gray et al., 1993; Pizza et al., 1996; Smith et al., 1996; van Eeden et al., 1999), with decreases also observed in the neutrophil adhesion marker CD62L following acute exercise (Kurokawa et al., 1995; van Eeden et al., 1999). This demonstrates the short-lived pro-inflammatory nature of acute exercise, but regular exercise has been shown to favour an anti-inflammatory environment at rest in healthy individuals.

1.7.2 Inflammatory mediators

1.7.2.1 Chronic responses

It is well established in a healthy population that regular exercise training and being physically active reduces systemic inflammation (Jankord & Jemiolo, 2004; Woods et al., 2012). This decrease in systemic inflammation is often characterised by reductions in circulating IL-6, IL-8, CRP, TNF- α , and fibrinogen concentrations following regular moderate aerobic exercise training (Beavers et al., 2010; Ernst, 1993; Johannsen et al., 2012; Nicklas et al., 2008; Febbraio, 2007; Gleeson, 2007; Handschin & Spiegelman, 2008; Pedersen & Febbraio, 2012), demonstrating the anti-inflammatory nature of exercise in healthy populations. The mechanisms underpinning these anti-inflammatory effects of exercise are not yet well established, but have been proposed to be a result of a reduction in cytokine production in fat and muscle tissue as part of the 'adaptive' response to exercise (Kasapis & Thompson, 2005; Ahmadizad, El-Sayed & Maclaren, 2006; Bodary et al., 2003; El-Sayed, Ali & El-Sayed, 2005; El-Sayed, Jones & Sale, 1999). Despite a lack of clarity around the capacity of exercise training to decrease basal inflammatory cell counts in healthy individuals, it is apparent that exercise training can provide anti-inflammatory benefits as demonstrated by a reduction of several markers of systemic inflammation.

1.7.2.2 Acute responses

In a healthy population, the acute inflammatory response to exercise is well established with an increase in inflammatory mediators post-exercise before returning to baseline levels 24 hours post-exercise (Peake et al., 2017; Woods et al., 2012). Strenuous acute exercise in healthy individuals has been seen to induce an increase in inflammatory mediators such as IL-6, IL-8, fibrinogen, CRP, TNF- α and markers for oxidative stress (e.g. increased ROS production contributing to

inflammation and muscular damage following maximal exercise) (Bizheh & Jaafari, 2011; Montgomery et al., 1996; Petersen & Pedersen, 2005). In particular, these responses can be seen during strenuous exercise (Ostrowski et al., 1999), and is characterised as an acute inflammatory response to exercise (Alessio, 1993; Pedersen & Hoffman-Goetz, 2000). However, there is still a lack of clarity with regards to CRP responses to acute exercise as a recent review categorised responses as 'inconclusive' (Brown et al., 2015). This mainly pro-inflammatory response is balanced by the increased release of anti-inflammatory cytokines (e.g. IL-10) which act to restrict the magnitude and duration of the inflammatory response (Northoff, Weinstock & Berg, 1994, Ostrowski et al., 1999; Pedersen & Hoffman-Goetz, 2000; Petersen & Pedersen, 2005). The extent of the increase in these factors yet again depends on both the intensity and duration of exercise (Reihmane et al., 2013).

When assessing these acute responses, it is important to consider a couple of factors. For example, it is believed that exercise training can blunt this acute response (Kasapis & Thompson, 2005; Mattusch et al., 2000; Montgomery et al., 1996), and training state needs to be considered as it is not yet clear whether sedentary individuals are more susceptible to an amplified inflammatory response when compared to trained individuals (Brown et al., 2015).

1.7.3 Respiratory symptoms

Whilst monitoring of respiratory symptoms are not characteristic of healthy adults there has been interest in the role of inflammatory parameters in reported symptoms following exercise. In healthy populations, exercise-induced changes in inflammatory parameters have been suggested to impact upon respiratory symptoms linked to infection. For example, heavy exercise training loads undertaken in a frequent manner have been linked with an acute increase in

respiratory symptoms (Nieman et al., 1990; Peters & Bateman, 1983). There have been proposals that increases in pro-inflammatory parameters and exercise-induced suppression of the immune system provides a mechanistic link for this increase in symptoms (Walsh et al., 2011). However, there is an underlying uncertainty as to whether these symptoms are a result of infection or rather inflammatory stimuli mimicking an URTI (e.g. exercise induced asthma/allergic airway inflammation) (Cox et al., 2010; Helenius, Lumme & Haahtela, 2005; Spence et al., 2007). There is little available evidence to link mechanisms with increased symptoms, but one proposal suggests that an enhanced migration of inflammatory cytokines into the airways as a result of exercise induced muscle damage may provide a potential mechanism (Peake, Nosaka & Suzuki, 2005; Pedersen et al., 2001).

1.8 Exercise as an anti-inflammatory intervention in COPD

Due to the established evidence in healthy populations, exercise immunology has progressed as a field to the stage where exercise can be considered as a potential form of anti-inflammatory therapy (Petersen & Pedersen, 2005; Timmons, 2005). Given the anti-inflammatory effects seen in healthy individuals, there is a strong rationale for assessing the immune response to exercise in individuals with inflammatory disease, especially COPD given the increasing evidence of the benefits of exercise on relevant clinical endpoints (i.e. exacerbations). An international respiratory statement previously highlighted the need to investigate the mechanisms behind exercise and inflammation in COPD to understand pro- and anti-inflammatory responses (Watz et al., 2014). This is of importance given that inflammation is considered a treatable trait in COPD and pharmacological therapies to date have been insufficient at tackling inflammation in COPD (Agusti et al., 2016).

1.8.1 Inflammatory cells

1.8.1.1 Chronic responses

There is limited evidence assessing inflammatory cell responses to chronic exercise training programmes in patients with COPD. Of the available evidence, previous research has suggested that a pulmonary rehabilitation programme does not induce significant reductions in blood total leukocyte and neutrophil counts (El Gammal et al., 2015; Mercken et al., 2005; Sciriha et al., 2017). However, these studies did not assess any markers of neutrophil function which have been shown in healthy individuals to be more sensitive to change with chronic exercise training programmes than counts alone. Mercken et al. (2005) demonstrated that 8 weeks of pulmonary rehabilitation was associated with reduced exercise-induced oxidative stress following an acute bout of exercise. This suggests that there is a chronic anti-inflammatory response to exercise in COPD. Therefore, the ability of exercise training to reduce oxidative stress in COPD whilst not enhancing inflammatory cell counts can be interpreted as a positive effect (Mercken et al., 2005). Further research is required to assess inflammatory cell counts and functional changes in response to pulmonary rehabilitation, especially in those susceptible to recurrent exacerbations to identify whether a cohort of patients experience an enhanced pro- or anti-inflammatory response with exercise training.

1.8.1.2 Acute responses

Leukocyte and neutrophil count response to acute exercise is well documented in healthy individuals with an acute mobilisation followed by a further delayed mobilisation, if the exercise is strenuous enough, in the hours following exercise before returning to resting levels 24 hours post-exercise (McCarthy & Dale, 1988; McCarthy et al., 1991). A similar inflammatory response to acute exercise has also recently been observed in COPD patients following an acute bout of interval walking

(Jenkins et al., 2015). Further evidence supports this by demonstrating that incremental exercise to symptom limitation in COPD patients induces short-term increases in blood neutrophils, lymphocytes and monocytes (Davidson et al., 2012; van Helvoort et al., 2005). In COPD, leukocytosis is observed at rest with maximal and submaximal exercise workloads inducing further elevations in circulating leukocytes, however the relative increase in leukocytes is not classified as exaggerated when compared to the inflammatory response seen in healthy individuals with exercise (Jenkins et al., 2015; van Helvoort et al., 2005; van Helvoort et al., 2006). It has, however, been suggested, that it is the activation state of neutrophils that requires further investigation in COPD and not the increased number, whereby neutrophils have been seen to have greater basal activation in COPD (Oudijk et al., 2005; Hoenderdos & Condliffe, 2013). Unfortunately, to date there is no available evidence assessing the range of neutrophil activation responses to exercise in patients with COPD. There remains a paucity of evidence examining leukocyte and neutrophil responses to acute exercise in COPD.

1.8.2 Inflammatory mediators

1.8.2.1 Chronic responses

Of the available evidence assessing changes in inflammatory mediators with exercise training in patients with COPD, utilising resistance or endurance training of moderate intensity for 8 weeks, research has suggested that the levels of plasma IL-6, IL-8, and CRP are not decreased following training (Ryrso et al., 2018). This is further supported by previous studies suggesting that 7-8 weeks of pulmonary rehabilitation does not modify the circulating levels of IL-6 and CRP (Canavan et al., 2007; El Gammal et al., 2015). However, recent evidence has cast doubt over this lack of an anti-inflammatory effect of exercise in COPD by showing that a moderate intensity exercise training programme is effective in reducing circulating

concentrations of CRP and IL-6 (Abd El-Kader, Al-Jiffri & Al-Shreef, 2016; de Alencar Silva et al., 2018).

In terms of physical activity levels, evidence suggests that COPD patients who are physically active have lower levels of circulating CRP and IL-6 (Moy et al., 2014). This is backed by further studies finding that physical inactivity is independently associated with elevated fibrinogen, CRP and TNF- α in COPD (Garcia-Aymerich et al., 2009; Waschki et al., 2012; Watz et al., 2008). Some have suggested that the mechanisms behind this have been proposed to be due to the lack of skeletal muscle use conferring systemic inflammation in inactive patients as opposed to the anti-inflammatory benefits of regular physical activity observed in active patients (Handschin & Spiegelman, 2008).

Despite conflicting findings, all the aforementioned studies do at least propose that exercise training and being physically active does not have any pro-inflammatory effects in COPD. For example, Canavan et al. (2007) concluded that pulmonary rehabilitation is unlikely to enhance systemic inflammation in non-muscle wasted COPD patients. This conclusion was also supported by studies of El Gammal et al. (2015) and Sciriha et al. (2017) who found that pulmonary rehabilitation did not mediate any pro-inflammatory markers in the average COPD patient. Further research is required to assess inflammatory responses to exercise training in patients in COPD, placing emphasis on patients with higher systemic inflammation (e.g. frequent exacerbators), and utilising established biomarkers for exacerbations (e.g. fibrinogen).

1.8.2.2 Acute responses

It has been proposed that markers such as IL-6 and CRP, which have been seen to be elevated in COPD (Gan et al., 2004; Wouters et al., 2007), could be key biomarkers to assess in response to exercise (de Alencar Silva et al., 2018; Garrod

et al., 2007; van der Vlist & Janssen, 2010). In terms of acute responses in COPD, studies have shown that acute exercise to symptom limitation does not induce significant changes in the concentrations of IL-6 or CRP (Canavan et al., 2007; Spruit et al., 2007; van Helvoort et al., 2005), whereas others have shown an increase in the concentration of IL-6 post-exercise in COPD patients (de Alencar Silva et al., 2018).

Taking this evidence, combined with that seen in healthy populations, it is important to assess the implications of pro-inflammatory responses traditionally observed with acute exercise, to determine whether this pro-inflammatory response could be detrimental in patients with higher systemic inflammation and increased susceptibility to infection (e.g. frequent exacerbators). For example, research has proposed that an exhaustive acute exercise bout could be detrimental for COPD patients due to increased exposure to ROS leading to bursts of acute inflammatory responses (van Helvoort et al., 2006). It is also important to examine the role exercise training could have on reducing this pro-inflammatory response as previous evidence has suggested that acute inflammatory responses to a single/acute bout of exercise have been seen to be blunted with exercise training (Gokhale, Chandrashekara, & Vasanthakumar, 2007). Further research is required to assess and interpret these inflammatory responses to acute exercise in patients with COPD, utilising markers of systemic inflammation (e.g. fibrinogen) which are heavily implicated in the prognosis of patients (e.g. exacerbation frequency) and have been left relatively unexplored.

1.8.3 Respiratory symptoms

In COPD, respiratory symptoms are elevated compared to healthy individuals with exercise training shown to reduce the burden of these symptoms (Spruit et al., 2016b). Exercise (pulmonary rehabilitation) has been shown to influence clinical

endpoints (risk of exacerbations (Moore et al., 2016)) but, like the evidence presented in healthy populations (e.g. respiratory infection), conclusive evidence of the mechanisms are not apparent yet. The anti-inflammatory nature of exercise training in COPD populations may play a role in this reduced reporting of respiratory symptoms (Jenkins, Holden & Jones, 2018). This evidence is based on chronic responses to exercise training but it is currently unclear to what extent respiratory symptoms may relate to responses to acute exercise. There have been calls for ongoing monitoring of respiratory symptoms in COPD patients undergoing exercise training (Franssen & Rochester, 2014). Further research is required to develop links between exercise and respiratory symptoms in COPD, especially in an acute setting whereby acute bouts of strenuous exercise may result in short bursts of inflammation which conceivably may increase (perception or incidence of) respiratory symptoms and/or exacerbation risk in COPD patients.

The effects of acute and/or chronic exercise on immune response in COPD are heavily understudied and the pro- and anti-inflammatory responses warrant investigation (Watz et al., 2014). It is also unclear how immune responses to exercise in COPD impact on disease activity (e.g. symptoms and exacerbations) and whether important clinical phenotypes display altered immune profiles in response to exercise.

1.9 Summary and Aims

COPD is a common, preventable and treatable disease characterised by persistent respiratory symptoms and largely irreversible and progressive airflow limitation that is due to airway and/or alveolar abnormalities induced by exposure to noxious stimuli. A distinct subset (phenotype) of patients are more susceptible to frequent exacerbations of COPD (a change in dyspnoea, cough and/or sputum production that is acute in onset and may warrant a change in regular medication) (Hurst et al.,

2010). Frequent exacerbators of COPD have heightened levels of local and systemic inflammation, which is considered a treatable trait (Agusti et al., 2016), and have been key targets of pharmacological approaches in this phenotype, with results so far proving unsuccessful.

Exercise, as part of pulmonary rehabilitation, is considered to be a cornerstone in the management of COPD, through improvements in dyspnoea, quality of life and exercise capacity (McCarthy et al., 2015). Research to date, has focussed on how exercise in COPD affects the overall population, not recognising potential differences between phenotypes, especially in terms of compliance to pulmonary rehabilitation. Research has also not addressed the mechanisms behind a reduction in exacerbations with pulmonary rehabilitation (Moore et al., 2016), and exercise alone in COPD (Jenkins et al., 2018). In a healthy population, it is well accepted that exercise confers anti-inflammatory and immune-modulatory properties which can also be seen in other chronic diseases associated with low-grade systemic inflammation (Petersen & Pedersen, 2005). There is limited research available addressing the effects of pulmonary rehabilitation, and exercise in general, on inflammation in frequent and infrequent exacerbators of COPD. Research is required to identify if anti-inflammatory effects of exercise are demonstrable in COPD patients, and whether such responses differ among clinical phenotypes, in order to inform whether non-pharmacological therapies such as pulmonary rehabilitation could also be used to target the treatable trait of systemic inflammation in frequent exacerbators of COPD.

The overall aim of this thesis is to assess the effects of exercise on clinical outcomes and inflammation and in frequent and infrequent exacerbators of COPD.

To do this, this thesis aims to address the following areas:

- Examine differences in pulmonary rehabilitation completion, clinical outcomes, and daily respiratory symptoms between frequent and infrequent exacerbators (Study 1)
- Assess resting inflammation following chronic exercise training as part of pulmonary rehabilitation in frequent and infrequent exacerbators (Study 2)
- Assess changes in corticosteroid inducible anti-inflammatory gene expression following pulmonary rehabilitation in frequent and infrequent exacerbators (Study 3)
- Assess inflammatory responses to acute exercise at the start and end of pulmonary rehabilitation in frequent and infrequent exacerbators (Study 4)
- Examine physical activity levels and inflammation in frequent and infrequent exacerbators of COPD following pulmonary rehabilitation (Study 5)

2 Chapter TWO

General Methods

2.1 Ethical Approval

The experimental procedures in this thesis received National Health Service (NHS) & Health Research Authority (HRA) approval via the London-Bromley (Research Ethics Committee (REC) Reference – 16/LO/0865) and West Midlands-Edgbaston (REC Reference – 18/WM/0081) REC's. Following NHS & HRA approval, procedures were locally approved through the College of Science REC (CoSREC), University of Lincoln (CoSREC Reference – CoSREC178; CoSREC Reference – CoSREC440). The procedures were fully compliant with; Declaration of Helsinki - Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Lincolnshire Community Health Services (LCHS) Countywide Community Respiratory Service (CCRS) Standard Operating Procedures, University of Lincoln College of Science Standard Operating Procedures, and University of Lincoln College of Science Risk Assessments. All patients & participants provided both verbal and written consent prior to involvement.

2.2 Inclusion Criteria

2.2.1 COPD patients (Studies 1 - 4)

COPD patients were included if they had been clinically diagnosed with any severity of COPD and had been referred to pulmonary rehabilitation.

2.2.2 Healthy comparators (Studies 2 & 3)

Healthy comparators were included if they had not been diagnosed with COPD or any other respiratory condition and were characteristically matched (age (between 45-85 years old) & smoking status (based on exhaled carbon monoxide content)) to the COPD patients.

2.3 Exclusion Criteria (Studies 1 - 4)

The exclusion criteria for COPD patients & healthy comparators were; inability or unwillingness to sign informed consent, any unstable ongoing cardiovascular events which may be exacerbated by exercise, inability to complete walk tests due to physical or mental impairment, other active inflammatory conditions e.g. rheumatoid arthritis or cancer, and confirmed asthma, allergic rhinitis or other respiratory disease (bronchiectasis, pulmonary fibrosis) as a primary or secondary diagnosis.

2.4 Recruitment

2.4.1 COPD patients (Studies 1 - 4)

COPD patients were first approached (as part of usual care) by the CCRS of the LCHS NHS Trust. Patients were contacted by an appropriate clinician (external to this study) to enrol onto pulmonary rehabilitation as part of standard procedures. At this point, potential participants received written and verbal information about study procedures. Patients consented or declined to being approached by the Chief Investigator at the initial assessment for pulmonary rehabilitation.

At initial assessment, patients were assessed by an appropriately qualified clinician (external to the study) for suitability to enrol onto pulmonary rehabilitation based on health status (supported by a health-screening questionnaire) and medical history. Clinicians also reviewed patient medications, mMRC dyspnoea score, comorbidities, and exacerbation history in the previous 12 months. Exacerbation events were based on patient recall with verification by medical records and defined as a respiratory event that led to a hospitalisation or the prescription of antibiotics and/or oral corticosteroids (Calverley et al., 2003). Spirometry measurements were obtained from patient records as part of a yearly GP review (in accordance with usual screening procedures of pulmonary rehabilitation). All of this information was

screened against study inclusion and exclusion criteria. These data were shared between LCHS NHS Trust and the University of Lincoln in linked anonymised form. If screened patients were deemed eligible and consented to being approached about taking part in the research study, they were referred onto the Chief Investigator at initial assessment for pulmonary rehabilitation. Procedures for each study were explained and further verification of inclusion and exclusion criteria was undertaken prior to obtaining written consent. Patients were made aware of their right to withdraw at any point without impacting upon the level of care they received. Following obtaining consent, patients provided information on age, smoking status/history, and comorbidities (presented using the Charlson Comorbidity Index (Charlson et al., 1987)) before providing measurements of: height, body mass and exhaled carbon monoxide (MicroCO Meter, CareFusion). Patients were then defined as either a frequent exacerbator or infrequent exacerbator based on reported exacerbation history in the previous 12 months. Frequent exacerbators were defined as having 2 or more exacerbations within the previous 12 months whilst infrequent exacerbators were defined as having no more than 1 exacerbation (Hurst et al., 2010). A flow diagram illustrating the recruitment of COPD patients for studies is detailed below (Figure 2.1).

2.4.2 Healthy comparators (Studies 2 & 3)

Healthy participants were screened in accordance with the inclusion/exclusion criteria and characteristics of pre-existing COPD recruits (e.g. age, gender and smoking status). The screening was undertaken by a nurse or General Practitioner (GP) based within the relevant GP Medical Practices (Nettleham Medical Practice & Lindum Medical Practice). Eligible participants were sent a study information pack (containing a cover letter, participant information sheet, consent form, declaration of interest form, and pre-paid envelope) by the nurse or GP. Participants who were

interested in taking part in the study completed and returned the declaration of interest form detailing their preferred method of contact (telephone or email) which was used by the Chief Investigator for recruiting participants. The initial contact between the Chief Investigator and participant involved the detailed explanation of study procedures and booking a visit time at their local GP practice. At the initial visit, participants had the opportunity to ask questions before providing verbal and written consent prior to study enrolment. During the visit, healthy participants were further screened in line with inclusion/exclusion criteria. Eligible healthy participants then provided verbal and written consent before collection of height, body mass, exhaled carbon monoxide reading (MicroCO Meter, CareFusion), current comorbidities (presented using the Charlson Comorbidity Index (Charlson et al., 1987)), spirometry (MicroLab Spirometer, MicroMedical), medications, and medical history. Healthy control participants then provided a single venous blood sample (section 2.7) before study participation was completed. The healthy comparator group did not partake in any intervention and were used for baseline comparisons in inflammation. The recruitment of healthy comparators for studies is detailed below (Figure 2.1).

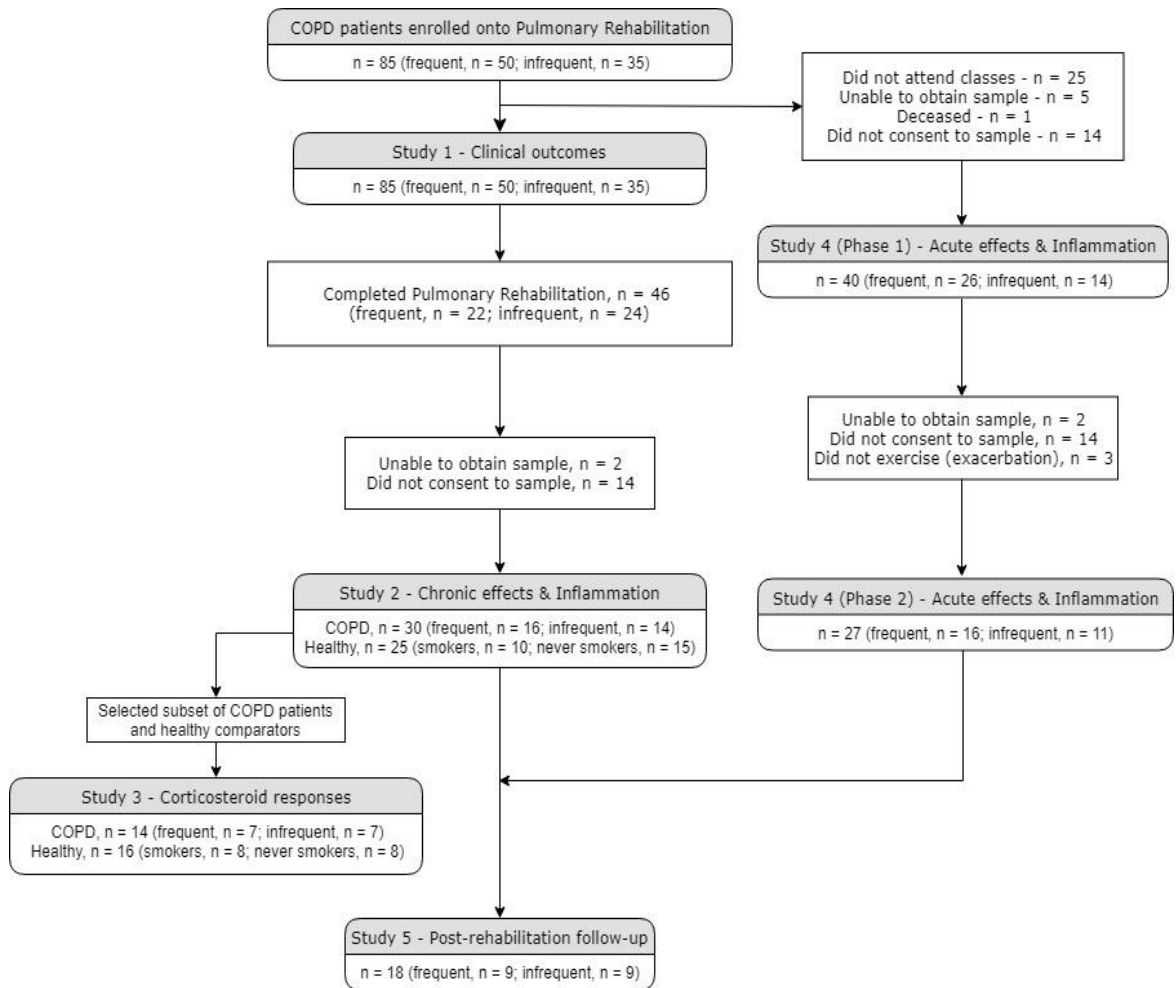


Figure 2.1. Flow diagram of COPD patients and healthy comparators throughout the studies comprising the thesis

2.5 Pulmonary Rehabilitation

Pulmonary rehabilitation was delivered by LCHS at two community venues (Sudbrooke Drive Community Centre & Bracebridge Community Centre) based in Lincoln. The pulmonary rehabilitation programme in Lincolnshire is delivered in accordance with NICE accredited BTS pulmonary rehabilitation guidelines (Bolton et al., 2013) and adheres to several quality standards as outlined in the UK National COPD Pulmonary Rehabilitation Audit in terms of programme content and length (Steiner et al., 2016). The pulmonary rehabilitation course, consisting of both exercise and education, was 8 weeks in duration with sessions taking place twice a week (16 sessions in total). Each session was 2 hr long with 1 hr devoted to exercise and 1 hr devoted to education in accordance with BTS guidelines (Bolton

et al., 2013). Patients were encouraged to partake in 1 further exercise session a week at home as part of the ongoing programme. Sessions were delivered by a fully qualified respiratory physiotherapist with the support of a respiratory physiotherapy assistant. These classes were specifically designed to improve exercise capacity and disease knowledge to help patients improve their management of COPD.

The exercise component of each session consisted of exercises aimed at improving cardiorespiratory fitness and muscle strength. Exercises aimed at improving cardiorespiratory fitness involved: walking, get up and go, and step-up exercises. Exercises aimed at improving muscular strength included: bicep curls, wall press, bent arm lateral raise, and cross and reach. Measurement of performance and perceived rating of exertion (modified Borg scale (Mahler & Horowitz, 1994)) on each exercise was assessed following each session to determine exercise targets for the next class in line with clinical judgement.

The accompanying education component included discussions of the following topics: introduction to COPD, nature of the disease, medications in COPD, bronchodilators & oxygen, managing exacerbations, chest clearance, breathing control, coping strategies 1 (physically), coping strategies 2 (psychologically), energy conservation, roles & benefits of exercise, diet & nutrition, relaxation therapy, benefits & allowances, previous patient perspective, and post-pulmonary rehabilitation options.

2.6 EXACT®

During initial assessment for pulmonary rehabilitation patients also had the opportunity to consent to filling in a daily respiratory symptoms questionnaire (EXACT®, EXACT-PRO initiative, Evidera). Patients filled this in via an online platform (Qualtrics Software, Utah, United States) or via a paper copy of the

questionnaire depending on availability of computer or smartphone access. EXACT[®] is a 14-item daily diary (score range 0-51) used to measure patient reported symptoms of exacerbations. Incorporated within this is an E-RS[™] 11 item subscale (score range 0-40) to measure daily respiratory symptoms. The E-RS[™] subscale is split into the following domains; Breathlessness (5 items, score range 0-17), Cough/Sputum (3 items, score range 0-11), and Chest (3 items, score range 0-12). Patients were instructed to fill in the questionnaire every evening for the week leading up to pulmonary rehabilitation commencement to provide a baseline measure in accordance with user guidelines (EXACT-PRO Initiative, Evidera). Patients were also asked to fill in the questionnaire for the first and last week of the pulmonary rehabilitation course to provide chronic (Study 1) and acute (Study 3) comparisons. A minimum of 4 data points per week were required for inclusion in the analysis in line with previous research (Leidy et al., 2014). This recorded data was used to provide a weekly average of respiratory symptoms to be used for analysis in line with EXACT[®] and E-RS[™] user guidelines (EXACT-PRO Initiative, Evidera). Patients reporting to have had an exacerbation at the beginning and/or end of pulmonary rehabilitation were excluded from analyses of EXACT[®] and E-RS[™] scores.

2.7 Blood collection (Studies 2 - 5)

Blood samples were collected in accordance with the standard operating procedures of the University of Lincoln and previous research (Jones et al., 2015). Participants were advised to remain seated performing minimal movement for 5 min prior to each blood sample with the exception of any post-exercise samples that were drawn within a few minutes of exercise cessation. Blood samples (~ 10 ml) were collected into Ethylenediaminetetraacetic acid (K₃EDTA) (6 ml) & Sodium Citrate (4.5 ml) treated vacutainers (Greiner Bio-One, Kremsmünster, Austria) from

the brachiocephalic vein (via standard venepuncture method), using a 21-gauge precision needle. A tourniquet was applied to the upper arm to establish the vein, before preparing the skin with an antibacterial alcohol swab. The tourniquet was maintained during the needle insertion process, and once blood flow was established within the second vacutainer, the tourniquet was released. All blood samples were maintained at room temperature to reduce the risk of potential temperature influences on cellular processing (Vogelaar et al., 2002).

2.8 Blood analysis

2.8.1 Total and differential blood leukocytes (Studies 2, 4, and 5)

An aliquot of whole blood (1 ml) from each EDTA-treated vacutainer was placed into a microcentrifuge tube for haematological analysis (total leukocyte, neutrophil, lymphocyte and eosinophil counts) using an automated haematology analyser (ABX Pentra 60C+ Haematology analyser, HORIBA Medical, Montpellier, France).

2.8.2 Inflammatory mediators (Studies 2, 4, and 5)

2.8.2.1 Fibrinogen

Circulating fibrinogen concentrations were measured via an enzyme-linked immunosorbent assay (ELISA) (Human Fibrinogen ELISA Kit, Assaypro LLC, Missouri, USA). Fibrinogen concentration was measured in Sodium Citrate treated blood. Following collection, blood was placed in a microcentrifuge tube for centrifugation at 2500 x g for 15 min at room temperature. Plasma was aliquoted into a new microcentrifuge tube before further centrifugation at 2500 x g for 15 min at room temperature. Plasma was then aliquoted (leaving the bottom 10% of sample) into a new microcentrifuge tube for storage at -80°C.

Before performing the ELISA, the plasma samples and ELISA reagents were left to thaw at room temperature. All steps of the ELISA including incubations were performed at room temperature in accordance with manufacturer instructions.

2.8.2.2 C-reactive protein

Circulating CRP concentrations were measured via ELISA (Human C-Reactive Protein ELISA Kit, Assaypro LLC, Missouri, USA). CRP concentration was measured in K₃EDTA treated blood. Following collection, remaining EDTA treated blood (see 2.8.1 and 2.8.4.1) was centrifuged at 1500 x g for 11 min at 4°C before aliquoting plasma into microcentrifuge tubes for storage at -80°C.

Before performing the ELISA, the plasma samples and ELISA reagents were left to thaw at room temperature. All steps of the ELISA including incubations were performed at room temperature in accordance with manufacturer instructions.

2.8.3 Flow cytometry (Studies 2 & 4)

An aliquot of whole blood (50 µl) from each EDTA-treated vacutainer was also used to measure the expression of established cell surface markers (CD11b, CD62L, CD66b) of neutrophils (CD45^{high}, CD16b^{low}) in COPD via flow cytometry (FACSVerse, Becton Dickinson (BD) Biosciences, New Jersey, USA) (Blidberg et al., 2012; Blidberg et al., 2013; Fortunati et al., 2009; Hoonhorst et al., 2014; Noguera et al., 2001). All blood samples were analysed via flow cytometry within 4 hrs of collection.

All conjugated monoclonal antibodies were titrated before use in analysis. Antibodies were titrated using a 5-step serial dilution with BD Pharmingen™ Stain Buffer (1:1) (BD, New Jersey, USA). For each antibody used to measure neutrophil expression, an isotype control was used in a separate tube. An additional unstained sample was also used to detect background signal alongside the isotype controls. Optimal mean fluorescent intensity signals were calculated against background signal to determine the optimum concentrations (Table 2.1).

Table 2.1. Dilution and concentrations of conjugated monoclonal antibodies

Marker	Fluorochrome	Volume	Dilution	Concentration
CD16b	PE	20 µl	1:2	0.125 µg
CD45	APC-H7	5 µl	1:4	0.250 µg
CD62L	BV421	5 µl	1:2	0.125 µg
CD66b	Alexa-Fluor 647	5 µl	1:4	0.063 µg
CD11b/Mac-1	BV510	5 µl	1:2	0.125 µg
Mouse Immunoglobulin M, κ Isotype control	Alexa-Fluor 647	5 µl	1:16	0.0625 µg
Mouse Immunoglobulin G1, κ Isotype control	BV510	5 µl	1:8	0.125 µg
Mouse Immunoglobulin G1, κ Isotype control	BV421	5 µl	1:8	0.125 µg

PE – R-Phycoerythrin, APC – Allophycocyanin, BV – Brilliant Violet

Once reagents were titrated, two tubes for each blood sample were prepared, one for the isotype control panel and one for the CD panel. The compatibility of antibodies and their corresponding fluorochromes were advised by BD for the optimisation of the panel. Each tube was prepared by first adding 50 µl Brilliant Stain Buffer (BD Biosciences, New Jersey, USA) to a falcon tube, before separately adding each diluted marker (CD panel: 20 µl CD16b, 5 µl CD45, 5 µl CD62L, 5 µl CD66b, 5 µl CD11b; Isotype control panel: 5 µl Alexa-Fluor 647, 5 µl BV510, 5 µl BV421). The Brilliant Stain Buffer was added before the conjugated monoclonal antibodies to reduce the risk of inappropriate binding of antibodies as per manufacturers recommendations. 50 µl of EDTA-treated whole blood was then added to each tube before gently mixing via vortex and incubating for 15 min in the dark at room temperature. After the incubation period, 450 µl of lysing solution (BD

FACS™ Lysing Solution 10X Concentrate diluted 1:10 with deionized water) was added and gently mixed again via vortex before incubating for a further 15 min in the dark at room temperature. Following incubation the sample was gently mixed again via vortex before analysis by flow cytometry (FACSVerse, Becton Dickinson, New Jersey, USA).

To identify neutrophil populations, the antibody panel was optimised using an assay with appropriate voltages to detect all cellular populations in forward-scatter and side-scatter plots. A performance quality control was performed each day on the FACS Verse before calibrating the assay to reduce the risk of errors in data. Granulocyte populations were identified using the gating strategy identified in Figure 2.2A resembling the light scatter characteristics of granulocytes as described previously (Im et al., 2011). The forward scatter component identifies cells based on size, but to isolate granulocytes from other white blood cells (e.g. monocytes), the side scatter is important as this identifies the granule component of granulocytes as seen by an increased intensity of side scatter (Shapiro, 1985). Neutrophils were identified from granulocytes by gating on CD16b⁺ and CD45⁺ cells as described in previous research (Figure 2.2B) (Cortjens et al., 2017), and recommended by the manufacturer.

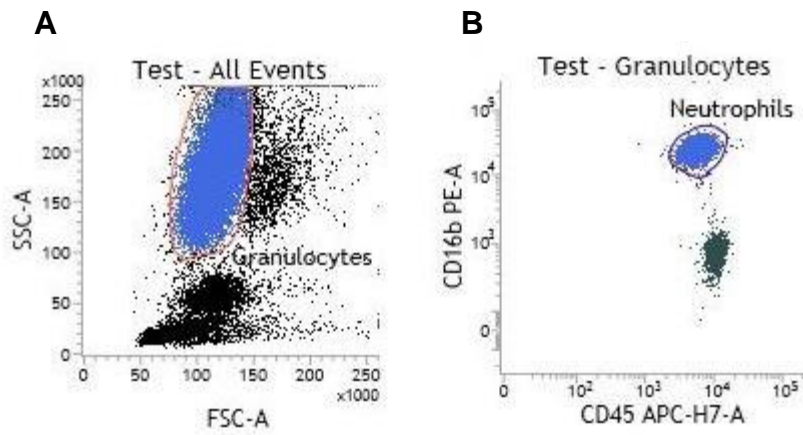


Figure 2.2. Gating strategies for identification of neutrophils via flow cytometry. a) Gating strategy for identifying granulocytes in whole blood using forward and side-scatter. b) Gating strategy for identifying neutrophils in the granulocyte population using CD45⁺ and CD16b⁺.

Neutrophil expression of activation markers (CD11b, CD62L and CD66b) was measured based on these events captured by the neutrophil gate (Figure 2.2B) using interval gates to measure differences between the isotype and CD panel (Figure 2.3). Median fluorescent intensity values of positive shifts in the expression of each marker (e.g. CD11b positive, CD62L positive, CD66b positive) were used for analysis.

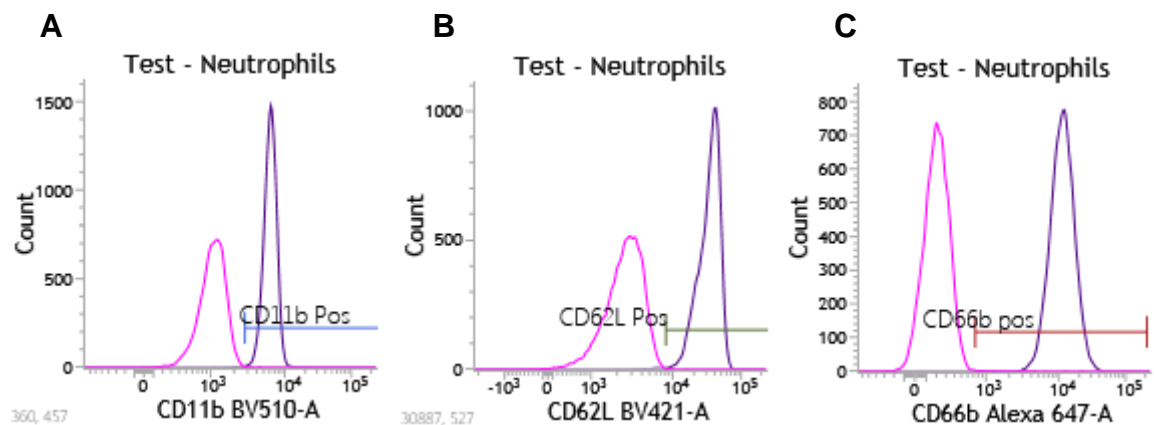


Figure 2.3. Interval gating strategy to detect shifts in CD11b (A), CD62L (B) and CD66b (C) expression between isotype controls and CD antibody panel.

Quad gates were also used to phenotype neutrophil populations (% of total cells) based on cellular maturity (mature neutrophils (CD16b^{high}/CD62L^{high}), immature cells (CD16b^{low}/CD62L^{high}), suppressive neutrophils (CD16b^{high}/CD62L^{low}), and progenitor neutrophils (CD16b^{low}/CD62L^{low})) by plotting CD62L against CD16b (Figure 2.4) in accordance with previous research (Cortjens et al., 2017). The percentage of total cells in each gate was extracted for analysis.

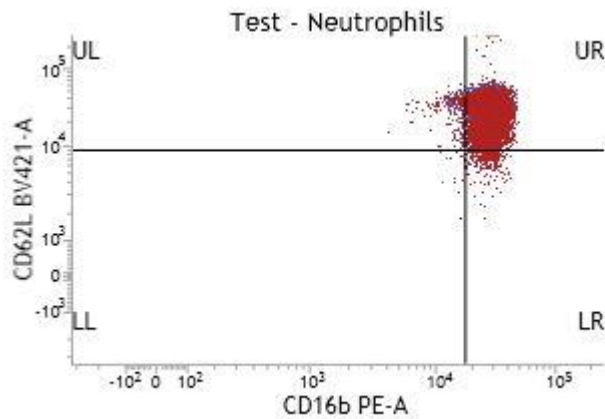


Figure 2.4. Quad gating strategy for identifying neutrophil phenotypes using CD16b and CD62L.

2.8.4 Anti-inflammatory gene expression (Studies 3 & 5)

2.8.4.1 PBMC isolation

For peripheral blood mononuclear cell (PBMC) isolation, 5 ml of K₃EDTA treated whole blood was carefully layered, with an automated pipette, on top of 3 ml of Mono-Poly Resolving Medium (MP Biomedicals, USA) in a 15 ml conical tube. The samples were then placed in a centrifuge (Centrifuge 5810R, Eppendorf, Hamburg, Germany) and spun for 45 min at 800 x g (room temperature) with deceleration (braking) set to the lowest possible setting to reduce cell band disturbance. Following centrifugation, plasma was carefully aliquoted and placed in a separate 15 ml conical tube for further centrifugation (see section 2.8.2.2). After removal of plasma, a sterile pasteur pipette was used to carefully remove the PBMC band before placing in a separate 15 ml conical tube.

Gibco™ Roswell Park Memorial Institute (RPMI) Medium 1640 (Life Technologies, California, USA) treated with streptomycin (100 µg/ml) and penicillin (100 units/ml) (5 ml solution) was added to the PBMC cell solution up to a volume of 10 ml before centrifugation for 5 min at 1200 x *g* (room temperature) with full braking applied. Following centrifugation, the RPMI 1640 medium was aspirated leaving a cell pellet at the bottom of each tube. A further 1 ml of RPMI 1640 medium was added to the cell pellet and thoroughly mixed to prepare a cell solution. For each tube, 10 µl of this cell solution was added to 10 µl of trypan blue stain and mixed in a microcentrifuge tube. This stained solution was then placed in a cell counting slide before analysis on an automated cell counter (TC20 Automated Cell Counter, Bio-Rad, USA). The automated cell counter was used to calculate a final concentration of live cells at 1×10^6 cells/ml. Once the dilutions were calculated, the required amount of RPMI 1640 medium was added to the original cell solutions and mixed.

2.8.4.2 PBMC treatment

Following dilution of the PBMC's to a working concentration, 200 µl of the cell solution was then aliquoted into a standard 96-well plate with 2 wells for each of the following treatment points; not-treated (NT), 2 hr treatment, and 6 hr treatment. The 96-well plates were then placed in an incubator overnight at 37°C and 5% carbon monoxide before treatment with 2 µl of dexamethasone (diluted 1:10 with 1640 RPMI Medium to make a concentration of 1µM) at selected time points (2 hr and 6 hr) the following day. Following treatment, the cell solutions were pipetted into microcentrifuge tubes and centrifuged for 5 min at 2500 x *g* at a temperature of 4°C. Following centrifugation, the supernatant was discarded, leaving a cell pellet which was mixed with 200 µl of RLT lysis buffer (Qiagen, Hilden, Germany) containing 1:100 diluted β-Mercaptoethanol (Sigma-Aldrich, Missouri, USA) before freezing at -80°C for later processing of RNA.

2.8.4.3 RNA extraction

RNA was extracted with the use of a Qiagen RNA extraction kit (RNeasy Mini Kit, Qiagen, Germany) with all centrifugation procedures taking place at room temperature. Samples were taken out of the -80°C freezer and allowed to thaw before the whole cell solution was pipetted into a genomic deoxyribonucleic acid (gDNA) eliminator column for centrifugation at 8000 x *g* for 30 s. The gDNA column was then discarded before 200 µl of 70% ethanol solution (diluted with RNase free water) was mixed gently with the flow through. The 70% ethanol and cell solution was then added to an RNA easy spin column and centrifuged at 8000 x *g* for 15 s. The flow through was this time discarded before a 3-step wash procedure was performed. The first wash procedure involved adding 700 µl of buffer RW1 (Qiagen, Germany) to the spin column before centrifugation at 8000 x *g* for 15 s. After discarding the flow through, this process was repeated but with 500 µl of buffer RPE (55ml RPE concentrate mixed with 4 volumes of 100% ethanol) (Qiagen, Germany). Finally, 500 µl of buffer RPE was again added to the spin column before centrifugation at 8000 x *g* for 2 min. After the 3-step wash was completed, the spin column was placed in a new collection tube and centrifuged for 1 min at maximum speed to dry out the RNA membrane. Following this, the spin column was placed in a fresh microcentrifuge tube and 30 µl of RNase-free water was applied directly to the spin column membrane before centrifugation at 8000 x *g* for 1 min. The spin column was then discarded and the microcentrifuge tube containing the RNA was frozen at -80°C in preparation for reverse transcription processing.

2.8.4.4 Reverse transcription

RNA samples were taken out of the -80°C freezer and allowed to thaw before RNA was reverse transcribed to complementary deoxyribonucleic acid (cDNA). 5 µl of RNA was aliquoted into 0.2 ml polymerase chain reaction (PCR) tubes and mixed

with 15 µl of mastermix consisting of the following; 4 µl of 5X variable input linear output (VILO) reaction mix, 2 µl of 10X Superscript enzyme mix (Superscript VILO mix, Invitrogen, California, USA) and 9 µl of RNase free water. The PCR tubes were then run through a thermal cycler (T100 Thermal Cycler, Bio-Rad, California, USA) using the following protocol; lid temperature of 105°C, solution volume of 20 µl, stage 1 at 25°C for 10 min, stage 2 at 42°C for 1 hr, stage 3 at 85°C for 5 min, and stage 4 where samples were permanently held at 12°C until removal from the thermal cycler. Once the thermal cycle was finished, 180 µl of RNase free water was added to each PCR tube and stored in the fridge at 4°C in preparation for PCR.

2.8.4.5 PCR

PCR's were performed using the following genes: Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (housekeeping gene), MKP-1, and GILZ which are both corticosteroid inducible genes (Newton & Holden, 2007).

2.5 µl of cDNA, or control standards, were mixed in a 96 well plate with 7.5 µl of a mastermix containing; 2.1 µl of RNase free water, 5 µl of Syber Green ER reaction mix (Invitrogen, California, USA), 0.2 µl of ROX reference dye (Invitrogen, California, USA), 0.1 µl forward primer (GAPDH, MKP-1 or GILZ), and 0.1 µl of reverse primer (GAPDH, MKP-1 or GILZ) (see Table 2.2 for primer sequences). Standard points consisted of diluted cDNA from the 2 hr and 6 hr treated samples with 25 µl of each sample placed into the first tube (S1). 180 µl of RNase free water was added to the remaining tubes (S2, S3, S4, S5) before taking 20 µl from S1 and mixing with S2, then 20 µl of S2 was taken into S3 and mixed, with this serial dilution repeated to tube S5. 180 µl of RNase free water was added to a tube assigned as water only (W) as the final standard point. Once all samples were mixed, the 96 well plates were sealed and scoured to secure each well individually before analysis using a StepOne Plus Real Time PCR System (Thermo-Fisher, USA). A standard

ramp speed test protocol was utilised (~2 hr) consisting of 10 min at 95°C before 40 cycles of 15 secs at 95°C followed by 1 min at 60°C. Following completion of the cycles, a melt curve was performed with incremental increases of 0.3°C from 60°C to 95°C. The 96 well plates were labelled according to samples and primers on the StepOne Software v2.3 (Thermo-Fisher, USA).

2.9 Statistical Analysis

Statistical analyses of all data were performed using the statistical computer software package Statistical Package for Social Sciences (SPSS) (v22.00; SPSS Inc., Chicago, IL, USA). Graphical representations of data were prepared using GraphPad Prism (v7; GraphPad Software Inc., California, CA, USA). All data were assessed for normal distribution prior to analysis using Shapiro-Wilk. If necessary, data were normalised (e.g. using log transformation) before further analysis. If data could not be normalised, a nonparametric equivalent was used. Statistical significance was accepted at $P < 0.05$.

Table 2.2. Primer sequences for GAPDH, MKP-1 and GILZ.

Gene	Primer Name	Accession Number	Sequence	Annealing temperature
GAPDH	GAPCF	NM_002046.3	TTCACCACCATGGAGAAGGC	60
	GAPCR		AGGAGGCATTGCTGATGATCT	60
MKP-1/DUSP1	MKP1RTF	NM_004417.3	GCTCAGCCTTCCCCTGAGTA	60
	MKP1RTR		GATACGCACTGCCCAGGTACA	60
GILZ/TSC22D3	GILZ F	NM_001015881.1 NM_004089.3	GGCCATAGACAACAAGATCG	60
	GILZR1	NM_198057.2	ACTTACACCGCAGAACCACCA	60

3 Chapter THREE

Study 1 – Clinical outcomes and respiratory symptoms in response to pulmonary rehabilitation in the frequent exacerbator phenotype of COPD

Abstract

Patients who suffer frequent exacerbations of COPD are recognised as a distinct clinical phenotype. Pulmonary rehabilitation is considered one of the most beneficial treatments for COPD inducing improvements in exercise capacity and quality of life. However, responses to pulmonary rehabilitation between frequent and infrequent exacerbators remain unclear. Based on exacerbation history in the previous year, 85 mild-very severe COPD patients (FEV_1 pred, $52 \pm 18\%$) were categorised as frequent (≥ 2 exacerbations) or infrequent exacerbators (≤ 1 exacerbation). The following clinical outcomes for pulmonary rehabilitation were analysed: completion rates, incremental shuttle (ISWT) & endurance shuttle walk tests (ESWT), chronic respiratory disease questionnaire (CRQ), hospital anxiety and depression scale (HADS). The Exacerbations of COPD tool (EXACT[®]) and Evaluating Respiratory Symptoms (E-RS[™]) measure were also completed daily for a week at the beginning and end of rehabilitation. Frequent exacerbators were less likely to complete pulmonary rehabilitation (44% vs 69%; $p=0.025$). Both groups experienced significant improvements in ISWT ($p<0.001$), ESWT ($p<0.001$) and quality of life measures (CRQ (all domains), $p<0.001$; Depression, $p=0.017$). Pulmonary rehabilitation significantly reduced total EXACT[®] ($p=0.009$) and E-RS[™] scores ($p=0.013$). Frequent exacerbators were less likely to complete pulmonary rehabilitation, but those who complete appear to experience similar benefits to infrequent exacerbators. Pulmonary rehabilitation should be encouraged in both frequent and infrequent exacerbators of COPD.

3.1 Introduction

Pulmonary rehabilitation is considered to be one of the most cost-effective options for treating COPD (Griffiths et al., 2001; Hoogendoorn et al., 2010; Mayers et al., 2007; Oba, 2007). The benefits of pulmonary rehabilitation are profound in the COPD population, especially when assessing outcomes such as exercise capacity and quality of life (Nici et al., 2006). However, not all patients who enrol onto, and complete pulmonary rehabilitation see the same benefits. Spruit et al. (2015a) have previously categorised four subsets of patients following pulmonary rehabilitation: 'very good responders', 'good responders', 'moderate responders' and 'poor responders'. This has led to proposals that clinical factors such as comorbidities or phenotypes might play an influential role in responses to pulmonary rehabilitation (Ambrosino et al., 2015; Hassan et al., 2016; Kaul, Farokhi & Megally, 2018).

One of the most established clinical phenotypes of COPD is the frequent exacerbator phenotype which has been shown to be relatively stable (Donaldson et al., 2013; Hurst et al., 2010). This phenotype is of significant clinical interest due to these patients having increased risk of hospitalisations and mortality (Steer, Gibson & Bourke, 2012). Frequent exacerbators also make more visits to their GP, receive more medication, and are associated with faster declines in lung function and health-related quality of life (Wedzicha, Mackay & Singh, 2013). As future exacerbations are best predicted by previous history of exacerbations, there are calls for therapies to target this phenotype (Vestbo & Lange, 2015).

Limited research has assessed responses to pulmonary rehabilitation between frequent and infrequent exacerbators of COPD. It has previously been shown that exacerbations are a risk factor for non-completion of pulmonary rehabilitation as these events have been associated with dropout or poor attendance at outpatient pulmonary rehabilitation (Fischer et al., 2009). It has also been shown that acute

exacerbations of COPD following pulmonary rehabilitation can have an acute detrimental effect on exercise capacity and quality of life (Carr, Goldstein & Brooks, 2007). However, Bohn et al. (2017) suggested that in patients who manage to complete outpatient pulmonary rehabilitation, improvements in exercise capacity can be seen to be larger in exacerbators compared to non-exacerbators. This improvement was measured by performance in the 6MWT with clinically significant improvements in exercise capacity observed in both groups but higher improvements were seen in frequent exacerbators. However, improvements in quality of life outcomes measured by the SGRQ were not significantly different between groups (Bohn et al., 2017). Another study also found that the subgroup of 'responders' to inpatient pulmonary rehabilitation, defined as having achieved the MCID for 6MWT, was made up of a higher percentage of frequent exacerbators compared to infrequent exacerbators (Zanini et al., 2013). This demonstrates that studies are currently limited in this area, especially utilising alternative clinical outcomes measures (e.g. ISWT and ESWT) and different settings (e.g. community-based), and provide conflicting findings creating uncertainty with regards responses to pulmonary rehabilitation between the exacerbator phenotypes.

The benefits of pulmonary rehabilitation stem beyond that of improvements in quality of life and exercise capacity. Another key benefit of pulmonary rehabilitation is a reduction in breathlessness (McCarthy et al., 2015; Ries et al., 2007). Respiratory symptoms are important outcomes to assess in the early detection of exacerbations (Beghe et al., 2013). Patients who experience frequent exacerbations have been seen to present with significantly higher mMRC dyspnoea scores (McGarvey et al., 2015). However, it is important to assess respiratory symptoms beyond breathlessness alone including chest tightness, cough and sputum production in order to provide a more comprehensive understanding of

COPD management, particularly in relation to exacerbation risk (Leidy & Murray, 2013). In the context of pulmonary rehabilitation, respiratory symptoms are often measured as part of a combined assessment of health status (e.g. CAT scores) (Bolton et al., 2013). However, this questionnaire relies on patient recall of respiratory symptoms over a period of time, not factoring in daily fluctuations in symptoms (Dodd et al., 2011). In COPD, respiratory symptoms can fluctuate significantly between days, and even within individual days, suggesting that patients are susceptible to under- or over-estimating their respiratory symptoms when filling in retrospective questionnaires (Kessler et al., 2006; Kessler et al., 2011; Partridge, Karlsson & Small, 2009). Further research also demonstrates this fluctuation in symptoms is accompanied with a steady progression of symptoms over time which has been closely related to the frequent exacerbator phenotype (Huerta et al., 2015)

Recently, the EXACT® questionnaire has been developed and validated to overcome limitations of previous tools and provide a standardised daily diary to assess exacerbations and respiratory symptoms in COPD (Leidy et al., 2010; Leidy et al., 2014). This 14-item questionnaire, with subscales monitoring specific respiratory symptoms, monitors daily respiratory symptoms in COPD and both the EXACT® and E-RS™ have been used in a number of clinical trials of pharmacological therapies, proving sensitive for detecting exacerbations and changes in respiratory symptoms (Beier et al., 2017; Papi et al., 2017; Tabberer et al., 2018). However, these patient reported outcomes have not yet been implemented in the context of pulmonary rehabilitation to assess respiratory symptoms upon enrolment and response to treatment across relevant clinical phenotypes.

This study aimed to assess the differences in clinical outcomes and daily reported respiratory symptoms pre- and post-pulmonary rehabilitation in frequent and infrequent exacerbators of COPD. It was hypothesised that frequent exacerbators would be: less likely to complete pulmonary rehabilitation, report greater respiratory symptoms but this would be reduced upon completion of pulmonary rehabilitation, and achieve comparable clinical benefit to infrequent exacerbators.

3.2 Methods

3.2.1 Participants

Eighty-five COPD patients enrolled on to pulmonary rehabilitation (age 69.5 ± 7.1 years; FEV₁ pred $52 \pm 18\%$; frequent exacerbators = 50; infrequent exacerbators = 35) between June 2016 and August 2018 and consented to take part in this study.

3.2.2 Pulmonary rehabilitation baseline and outcome assessment

Prior to attendance at initial assessment, participants were required to complete the HADS (Zigmond & Snaith, 1983) (anxiety and depression domains) and CRQ (Williams et al., 2003) (dyspnoea, emotion, fatigue, and mastery domains).

The participants then performed 3 walk tests (2 x ISWT, 1 x ESWT) to determine exercise capacity and tolerance. These walk tests were conducted in accordance with European Respiratory Society/American Thoracic Society field test guidelines (Holland et al., 2014). Each test was separated by at least 20 min to allow sufficient time for recovery. Patients first performed an ISWT on a flat, level surface within a community hall. Patients were instructed to walk to and around cones that were 9 metres apart in time with the bleeps. The walk test was ended when patients were unable to make the cone before the bleep sounded or, if patients requested to stop, or if the physiotherapist noticed a clear deterioration in health. Following the rest period and completion of the repeated ISWT, the furthest distance achieved between tests was used to calculate the level to be used on the ESWT in

accordance with test guidelines (Revill et al., 1999). The ESWT was performed using the procedures outlined for the ISWT.

The questionnaires and walk tests were repeated at the final assessment following completion of the community pulmonary rehabilitation programme. Completion of community pulmonary rehabilitation was defined as attendance at 12 or more sessions in accordance with previous research (Boutou et al., 2014), and national recommendations for pulmonary rehabilitation (Department of Health, 2012b). Clinical outcome data were only included if patients met the outlined criteria for completion.

3.2.3 EXACT®

At initial assessment patients consented to filling in the daily EXACT® questionnaire (EXACT-PRO Initiative, Evidera). Questionnaire delivery was determined via patient preference (see section 2.6). Patients were instructed to complete the questionnaire daily in the week leading up to the beginning of pulmonary rehabilitation to provide a baseline measure. Patients then completed the questionnaire again for each of the last seven days of pulmonary rehabilitation to provide an end of rehabilitation measure. Any patients who reported to have had an exacerbation during pulmonary rehabilitation were removed from this specific analysis.

3.2.4 Statistical Analysis

Data on the proportion of patients within each group (frequent vs infrequent exacerbators) who did not complete pulmonary rehabilitation was reported using chi-squared analysis. Clinical outcomes (ISWT, ESWT, HADS, CRQ), mean EXACT® scores, and mean E-RS™ subscale (including individual domains; Breathlessness, Cough/Sputum, and Chest) were analysed using a two-way mixed Analysis of Variance (ANOVA) with Bonferroni correction to assess changes

between frequent and infrequent exacerbators pre- and post-pulmonary rehabilitation. The proportion of participants in each group (frequent and infrequent exacerbators) to achieve MCID's in the following outcomes: ISWT ($\uparrow 47.5\text{m}$) (Jones et al., 2014a), ESWT ($\uparrow 154\text{m}$) (Altenburg et al., 2015), HADS (anxiety, $\downarrow 1.3$ points; depression, $\downarrow 1.5$ points) (Smid et al., 2017), CRQ ($\uparrow 0.5$ point average) (Jones et al., 2014a), and E-RS™ (overall, $\downarrow 3$ points; breathlessness, $\downarrow 2$ points; cough/sputum, $\downarrow 1$ point; chest, $\downarrow 1$ point) (Yang et al., 2018), were also compared using chi-squared analysis. Statistical significance was accepted at $P < 0.05$.

3.3 Results

3.3.1 Patient characteristics

The patient characteristics of frequent and infrequent exacerbators are detailed in Table 3.1. All frequent exacerbators were categorised as GOLD grade D (100%) with the majority of infrequent exacerbators classified as GOLD grade B (89%). The majority of frequent and infrequent exacerbators were categorised as either mMRC grade 2, grade 3, or grade 4.

Table 3.1. Patient characteristics

Variable	Frequent	Infrequent
Age (years) ^a	69.6 ± 7.0	69.3 ± 7.3
% Males ^b	58%	57%
Body Mass (kg) ^a	77.3 ± 17.7	83.0 ± 18.9
GOLD grade, n (%) ^c		
A	0 (0)	4 (11)
B	0 (0)	31 (89)
C	0 (0)	0 (0)
D	50 (100)	0 (0)
FEV ₁ %predicted ^a	48 ± 16	57 ± 20
Charlson Comorbidity Index ^a	4.2 ± 1.3	4.2 ± 1.2
Current smokers ^b	32%	23%
mMRC, n (%) ^c		
0	0 (0)	2 (6)
1	0 (0)	2 (6)
2	3 (6)	7 (20)
3	16 (32)	9 (25)
4	31 (62)	15 (43)

^aData presented as mean ± SD. ^bData presented as a % of total population. ^cData presented as total number (% of group). Frequent, n = 50; infrequent, n = 35.

3.3.2 Completion rates

Of those who consented to take part in the study, 54% of COPD patients completed pulmonary rehabilitation (frequent exacerbators, $n = 22$; infrequent exacerbators, $n = 24$). Frequent exacerbators were significantly less likely to complete pulmonary rehabilitation when compared to infrequent exacerbators (44% vs 69%; $p = 0.025$) (Figure 3.1).

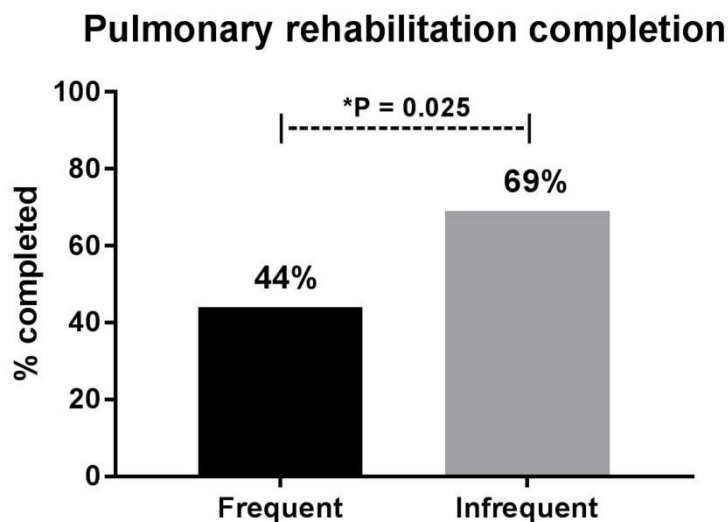


Figure 3.1. Pulmonary rehabilitation completion rates between frequent and infrequent exacerbators. Data presented as % of group to attend 12 or more pulmonary rehabilitation classes.*Significant difference between groups ($p < 0.05$).

3.3.3 Clinical outcomes

3.3.3.1 Exercise capacity

A subset of COPD patients did not undertake either the ISWT (frequent, $n = 1$; infrequent, $n = 4$) or ESWT (frequent, $n = 2$; infrequent, $n = 5$) during initial assessment for pulmonary rehabilitation meaning exercise capacity data were not available for these patients.

A significant main effect of time was observed showing improvements in exercise capacity in both frequent and infrequent exacerbators (ISWT, $p < 0.001$; ESWT, $p < 0.001$). Both groups met the MCID for ESWT ($> \uparrow 154\text{m}$), but only the frequent exacerbators group as a whole met the MCID for ISWT (frequent, $\uparrow 61\text{m}$; infrequent,

↑41m). Chi-squared analysis demonstrated a larger proportion of infrequent exacerbators met the MCID for improvement on the ESWT when compared to frequent exacerbators (29% vs 60%; $p = 0.038$). No significant differences were observed in the proportion of patients in each group who met the MCID for ISWT with chi-squared analysis (62% vs 45%; $p = 0.278$). The ANOVA found no independent effects of group (ISWT, $p = 0.860$; ESWT, $p = 0.276$), nor were there any indications of a time \times group interaction (ISWT, $p = 0.390$; ESWT, $p = 0.207$) (Table 3.2).

Table 3.2. Walk test outcomes pre- and post-rehabilitation

			p-values		
			Time		Group
			main	Time \times group	main
Walk Test	Frequent	Infrequent	effect	interaction	effect
ISWT (m)			<0.001*	0.390	0.860
Pre-Rehab	258 \pm 149	262 \pm 124			
Post-Rehab	319 \pm 147	303 \pm 121			
ESWT (m)			<0.001*	0.207	0.276
Pre-Rehab	347 \pm 522	327 \pm 325			
Post-Rehab	585 \pm 588	794 \pm 521			

Data presented as mean \pm SD. *Significant main effect of time ($p < 0.001$). ISWT: frequent, $n = 21$; infrequent, $n = 20$. ESWT: frequent, $n = 20$; infrequent, $n = 19$.

3.3.3.2 Quality of life

A significant main effect of time showing improvements in all domains of the CRQ were observed post-rehabilitation with MCID's met in both frequent and infrequent exacerbators ($p < 0.001$). Chi-squared analysis demonstrated that there were no

significant differences in terms of proportion of frequent and infrequent exacerbators to meet the MCID for improvement on the CRQ (50% vs 71%; $p = 0.091$). There were no significant group effects on absolute changes in scores of CRQ subdomains (Dyspnoea, $p = 0.899$; Emotion, $p = 0.861$; Fatigue, $p = 0.291$; Mastery, $p = 0.345$). No time \times group interactions were observed in any of the CRQ subdomains between frequent and infrequent exacerbators (Dyspnoea, $p = 0.453$; Emotion, $p = 0.452$; Fatigue, $p = 0.206$; Mastery, $p = 0.837$) (Table 3.3).

3.3.3.3 Anxiety & Depression

A significant main effect of time was observed showing a reduction in depression scores following pulmonary rehabilitation in both frequent and infrequent exacerbators ($p = 0.017$). However, anxiety ($p = 0.197$) was not significantly reduced as a result of pulmonary rehabilitation. The change in anxiety following pulmonary rehabilitation within infrequent exacerbators ($\downarrow 1.38$ points) did meet the MCID. MCID's were not reached for depression in either group as a whole. Chi-squared analysis showed that no significant differences were observed in the proportion of patients in each group who met the MCID for anxiety (36% vs 46%; $p = 0.685$) and depression (45% vs 50%; $p = 0.979$). The ANOVA found no significant group effects between frequent and infrequent exacerbators (Anxiety, $p = 0.503$; Depression, $p = 0.348$), nor were any time \times group interactions observed (Anxiety, $p = 0.142$; Depression, $p = 0.538$) (Table 3.3).

Table 3.3 CRQ and HADS outcomes following pulmonary rehabilitation

Domains	Frequent	Infrequent	p-values		
			Time main effect	Time × group interaction	Group main effect
Dyspnoea score			<0.001**	0.453	0.899
Pre-Rehab	2.60 ± 1.28	2.45 ± 1.05			
Post-Rehab	3.58 ± 1.43	3.74 ± 1.14			
Emotion score			<0.001**	0.452	0.861
Pre-Rehab	4.52 ± 1.37	4.34 ± 1.30			
Post-Rehab	4.98 ± 1.09	5.04 ± 1.17			
Fatigue score			<0.001**	0.206	0.291
Pre-Rehab	3.70 ± 1.34	3.83 ± 1.42			
Post-Rehab	4.09 ± 1.12	4.66 ± 1.06			
Mastery score			<0.001**	0.837	0.345
Pre-Rehab	4.34 ± 1.58	4.67 ± 1.43			
Post-Rehab	4.99 ± 1.29	5.38 ± 1.10			
Anxiety score			0.197	0.142	0.503
Pre-Rehab	6.45 ± 4.79	7.96 ± 4.32			
Post-Rehab	6.55 ± 3.61	6.58 ± 4.04			
Depression score			0.017*	0.538	0.348
Pre-Rehab	6.09 ± 4.36	7.33 ± 4.56			
Post-Rehab	5.41 ± 3.83	6.00 ± 4.30			

Data presented as mean ± SD. *Significant main effect of time (* p < 0.05, ** p < 0.001). Frequent, n = 22; infrequent, n = 24.

3.3.4 EXACT® and E-RS™

A subset of patients (frequent, n = 14; infrequent, n = 16) provided sufficient daily EXACT® data in the week prior to pulmonary rehabilitation and in the final week of the course. There were 3 patients who experienced an exacerbation in the frequent group and 2 in the infrequent group leaving 11 frequent and 14 infrequent exacerbators in the analysis.

Pulmonary rehabilitation significantly reduced the average EXACT® weekly score in both frequent and infrequent exacerbators ($p = 0.009$). There was a tendency towards a time \times group interaction with EXACT® scores but this was not found to be statistically significant ($p = 0.065$). There were no significant differences between groups for average weekly EXACT® score ($p = 0.120$) (Table 3.4).

The E-RS™ domain was significantly reduced following pulmonary rehabilitation in both frequent and infrequent exacerbators ($p = 0.013$). No significant time \times group interactions were observed ($p = 0.104$), nor were there any significant differences between groups for the E-RS™ domain ($p = 0.189$) (Table 3.4). Chi-squared analysis showed no significant differences in the proportion of patients in each group who met the MCID for the E-RS™ Total (45% vs 36%; $p = 0.622$).

Pulmonary rehabilitation significantly reduced scores in the Breathlessness domain ($p = 0.023$), with a tendency towards a similar reduction in the Chest domain ($p = 0.060$). There were no significant reductions observed in the Cough/Sputum domain following pulmonary rehabilitation ($p = 0.136$). There was a tendency towards a significant time \times group interaction in the Cough/Sputum domain but this was not found to be statistically significant ($p = 0.074$). There were no significant time \times group interactions in the Breathlessness ($p = 0.232$) and Chest domains ($p = 0.270$) with pulmonary rehabilitation. There were also no significant differences between groups across all of the symptom specific domains (Breathlessness, $p =$

0.263; Cough/Sputum, $p = 0.288$; Chest, $p = 0.357$) (Table 3.4). Chi-squared analysis demonstrated no significant differences in the proportion of patients in each group who met the MCID for the: Breathlessness (36% vs 29%; $p = 0.678$), Cough/Sputum (27% vs 14%; $p = 0.420$), and Chest domains (45% vs 29%; $p = 0.383$).

Table 3.4. Patient reported EXACT® questionnaire scores

EXACT® domain	Frequent	Infrequent	p-values		
			Time main effect	Time x group interaction	Group main effect
EXACT®-Total			0.009*	0.065	0.120
score					
Pre-Rehab	21.7 ± 8.7	15.6 ± 6.5			
Post-Rehab	17.4 ± 6.5	12.6 ± 5.3			
E-RS™-Total			0.013*	0.104	0.189
score					
Pre-Rehab	17.7 ± 6.5	13.6 ± 5.7			
Post-Rehab	13.8 ± 4.8	12.7 ± 4.2			
Breathlessness			0.023*	0.232	0.263
score					
Pre-Rehab	9.0 ± 4.1	7.1 ± 3.2			
Post-Rehab	7.5 ± 3.0	6.6 ± 2.5			
Cough/Sputum			0.136	0.074	0.288
score					
Pre-Rehab	3.2 ± 0.7	2.5 ± 1.1			
Post-Rehab	2.5 ± 1.0	2.6 ± 0.6			
Chest			0.060	0.270	0.357
score					
Pre-Rehab	3.7 ± 2.0	2.7 ± 1.7			
Post-Rehab	2.6 ± 2.3	2.4 ± 1.7			

Data presented as mean ± SD. Frequent, n = 11; infrequent, n = 14. *Significant main effect of time (* p < 0.05, ** p < 0.001).

3.4 Discussion

The main findings from this study suggest that frequent exacerbators are less likely to complete pulmonary rehabilitation compared to infrequent exacerbators but in those who complete pulmonary rehabilitation, they on average experience similar absolute changes in clinical outcomes compared to infrequent exacerbators.

There is a depth of research assessing how exacerbations may predict completion of pulmonary rehabilitation in a mixed cohort but a paucity of studies have prospectively investigated the frequent exacerbator phenotype in pulmonary rehabilitation. The lower completion rates in frequent exacerbators observed in the present study may relate to previous evidence where exacerbations have been reported as a prominent cause of poor uptake and completion of pulmonary rehabilitation (Fischer et al., 2009; Hayton et al., 2013; Jones et al., 2014b; Keating, Lee & Holland, 2011). The average completion rate for the current study was 54%, which is lower than the national average (62%) reported in the recent UK National COPD Pulmonary Rehabilitation Audit (Steiner et al., 2018). This would suggest that pulmonary rehabilitation completion rates are poorer in Lincoln compared to that observed nationally in the COPD audit (Steiner et al., 2018). Frequent exacerbators were observed to have completion rates 18% lower than the national average (44% vs 62%) suggesting that the frequent exacerbator phenotype may be contributing more than infrequent exacerbators to this poor completion rate (Steiner et al., 2018). Although this study suggests frequent exacerbators are less likely to complete pulmonary rehabilitation, this study did not provide evidence on the specific reasons for drop-out which may directly or indirectly be related to incidence of exacerbations (e.g. transportation issues) (Hayton et al., 2013; Keating, Lee & Holland, 2011). Future larger scale studies are required assessing the frequent exacerbator phenotype and completion rates. Future studies should also gather

data on reasons for drop-out to see whether the increased susceptibility to recurrent exacerbations in this phenotype is a factor in poor completion of pulmonary rehabilitation.

This study has shown that frequent exacerbators benefit from pulmonary rehabilitation in a similar manner to infrequent exacerbators. Improvements in traditional outcomes such as: ISWT, ESWT, CRQ and Depression were observed in both groups agreeing with previous literature in the COPD population (McCarthy et al., 2015). These findings also agree with those of Bohn et al., (2017), Steele et al., (2010), and Zanini et al., (2013) showing that frequent exacerbators can experience significant benefits as a result of pulmonary rehabilitation, although the current study was unable to suggest that frequent exacerbators respond better to pulmonary rehabilitation than infrequent exacerbators as seen in the aforementioned studies. However, it is important to note that acute exacerbations in the post-rehabilitation setting in COPD patients who complete pulmonary rehabilitation can have clinically significant reductions in exercise capacity and quality of life outcomes (Carr, Goldstein & Brooks, 2007). Furthermore, the proportion of patients in each group who met the MCID for the aforementioned outcomes was also similar barring the ESWT outcome where infrequent exacerbators were more likely to meet the MCID. However, our findings also suggest that only the frequent exacerbators group as a whole met the MCID for improvement in ISWT. It is important to note that despite no statistically significant reductions in anxiety being observed, contrary to previous findings (McCarthy et al., 2015), only infrequent exacerbators as a whole met the MCID for anxiety and neither group met the MCID for depression. It has, however, been shown that reductions in anxiety and depression are largely observed in COPD patients presenting with severe symptoms (e.g. >11 points) (Harrison et al., 2012), and that

the effect of pulmonary rehabilitation can often be underestimated when utilising patients with mild symptoms as observed in the current study. Taken together, the available evidence suggests COPD patients achieve benefits in the majority of traditional clinical outcomes from completion of pulmonary rehabilitation irrespective of exacerbation history, but further research is required assessing the magnitude of change, particularly in achieving MCID's for ISWT, ESWT and HADS.

To date, this is the first study to utilise the recently validated EXACT[®] tool in the context of pulmonary rehabilitation for monitoring exacerbations and respiratory symptoms. This study found that pulmonary rehabilitation was effective in reducing total EXACT[®] scores, E-RS[™] domain scores and breathlessness scores. These findings using the EXACT[®] tool agree with previous research utilising the CAT showing that pulmonary rehabilitation is effective in reducing respiratory symptoms (Dodd et al., 2011). The EXACT[®] questionnaire was designed to standardise the assessment of respiratory symptoms including daily fluctuations (Leidy & Murray, 2013), rather than provide respiratory symptoms based on patient recall at isolated timepoints as seen with the CAT (Dodd et al., 2011). However, it is important to note that only a subset of patients in this study completed the questionnaire due to feasibility issues. For example, willingness to use and access internet platforms were a common issue with some patients opting to complete the questionnaire via paper. This may again highlight the challenges of utilising technology in an older population (Deng, Mo & Liu, 2014). The use of a paper copy of this questionnaire, however, introduces elements of bias and is not the preferred mode of delivery in the guidance (EXACT-PRO Initiative, Evidera), but issues with internet availability and general questionnaire compliance resulted in the introduction of this format. Therefore, future research is warranted assessing the feasibility of monitoring respiratory symptoms utilising the EXACT[®] tool throughout the duration of the

rehabilitation course and draw comparisons between frequent and infrequent exacerbators.

There are some important factors to consider when interpreting the results of this study. Although there were lower completion rates in the frequent exacerbator group, there may be contributing factors that were not considered in this study that have recently been identified to be linked to poor completion (e.g. frailty, social deprivation) (Maddocks et al., 2016; Steiner et al., 2017b). The effects of these factors cannot be dismissed, but the aim of the current study was to identify how the frequent exacerbator phenotype relates to response to pulmonary rehabilitation. Depression and smoking status are other key recognised barriers for non-completion (Hayton et al., 2013; Keating, Lee & Holland, 2011). However, the current study observed comparable characteristics across groups in terms of proportion of smokers, depression and other characteristics (e.g. number of comorbidities). The EXACT[®] analyses must also be interpreted with caution as these findings only included patients who remained clear of exacerbations during pulmonary rehabilitation. This study also excluded COPD patients with co-existing lung conditions which must be factored in when interpreting the results of the study. This was undertaken to ensure the exacerbation history was related to exacerbations of COPD and not that of other lung conditions.

In conclusion, frequent exacerbators were less likely to complete pulmonary rehabilitation compared to infrequent exacerbators. Frequent exacerbators can expect to see the same benefits in terms of improvements in exercise capacity and quality of life measures. Pulmonary rehabilitation was also effective in reducing respiratory symptoms in frequent and infrequent exacerbators as measured by the newly validated EXACT[®] tool. Further research is required assessing responses to

pulmonary rehabilitation among the frequent exacerbator phenotype and assessing the feasibility of the EXACT[®] tool in the context of pulmonary rehabilitation.

4 Chapter FOUR

Study 2 – The chronic effects of pulmonary rehabilitation on inflammation and immunity in frequent and infrequent exacerbators of COPD

Abstract

Frequent exacerbators of COPD have been suggested to have heightened systemic inflammation. Exercise in healthy populations has been proposed to confer anti-inflammatory properties. The aim of this study was to see whether pulmonary rehabilitation modulates markers of systemic inflammation in frequent and infrequent exacerbators of COPD. Blood samples were analysed from 30 mild-very severe COPD patients (FEV₁ pred, 52 ± 17%; frequent, n = 16; infrequent, n = 14) at the start and end of pulmonary rehabilitation. The primary outcome was fibrinogen concentration. Secondary outcomes included: CRP concentration, total and differential leukocyte counts, neutrophil activation (CD11b, CD62L & CD66b), and neutrophil maturity (mature, immature, suppressive, progenitor). Pulmonary rehabilitation significantly reduced the resting concentrations of fibrinogen (p = 0.033). Reductions in total leukocyte (p = 0.018) and blood neutrophil counts (p = 0.018) were observed at the end of pulmonary rehabilitation in frequent exacerbators only. Pulmonary rehabilitation did not significantly reduce the resting concentration of plasma CRP (p = 0.937) or blood neutrophil expression of CD11b (p = 0.553), CD62L (p = 0.070), or CD66b (p = 0.317). Pulmonary rehabilitation significantly reduced the percentage of progenitor neutrophils in both groups (p = 0.015). None of the other neutrophil subsets were significantly affected by pulmonary rehabilitation (mature, p = 0.313; immature, p = 0.756; suppressive p = 0.259). This study suggests that pulmonary rehabilitation has the potential to modify surrogate markers of exacerbation risk in COPD and reduce levels of systemic inflammation in frequent exacerbators. Further studies are required to

explore the potential anti-inflammatory effects of exercise in COPD and its phenotypes utilising both pro- and anti-inflammatory markers.

4.1 Introduction

As established in Study 1, frequent exacerbators are less likely to complete pulmonary rehabilitation. Failure to complete pulmonary rehabilitation is an adverse indicator for hospital admission (Moore et al., 2016; Steiner et al., 2017a). Those who complete pulmonary rehabilitation have been seen to have a lower risk of experiencing an exacerbation or hospitalisation admission (Moore et al., 2016). However, the mechanisms underpinning a reduced incidence of hospital admissions in those who complete pulmonary rehabilitation are unclear.

Frequent exacerbators display an altered immune profile that is considered to provide the potential biological underpinning of recurrent exacerbation events (Wedzicha, Mackay & Singh, 2013). Systemic inflammatory parameters such as; fibrinogen, CRP, leukocyte, and blood neutrophil counts have been seen to be elevated in frequent exacerbators (Donaldson et al., 2005a; Hurst et al., 2010; Wedzicha, Mackay & Singh, 2013). A variety of pharmacological therapies have been shown to be ineffective or have conflicting results in relation to arresting long-term persistent systemic inflammation in COPD (King, 2015). The treatment of inflammation in COPD does not currently stem beyond that of pharmacological treatment, with the effects of non-pharmacological treatments left relatively unexplored.

Recently, Jenkins et al. (2018), (section 1.6.2) synthesised the available evidence on maintenance supervised exercise programmes following pulmonary rehabilitation and found further reductions in incidence of exacerbations and hospitalisations compared to pulmonary rehabilitation alone. Such independent effects of exercise interventions on exacerbation risk may suggest that there are unexplored mechanisms (Jenkins, Holden & Jones, 2018), compared to previously proposed indirect effects (e.g. changes in dyspnoea, physical conditioning and

enhanced disease knowledge (Evans & Steiner, 2018)). One potential area is that of exercise as an immunomodulator, based on extensive evidence of the anti-inflammatory effects of exercise in healthy (including aged) individuals (Woods et al., 2012). However, questions remain around the potential for such mechanisms in COPD with authors claiming that it is not plausible for interventions such as pulmonary rehabilitation to affect inflammatory or infectious events directly (Evans & Steiner, 2018).

Previous research has suggested that pulmonary rehabilitation does not affect systemic inflammation as characterised by a lack of changes in plasma CRP, plasma cytokine concentrations or total blood leukocyte count even in the presence of improved exercise capacity (Canavan et al., 2007; Sciriha et al., 2017). Chronic exercise training programmes, similar in length to pulmonary rehabilitation have been seen to reduce systemic inflammation (e.g. CRP) in other clinical (e.g. type 2 diabetes) populations (Giannopoulou et al., 2005). Furthermore, walking exercise programmes have been seen to have anti-inflammatory effects in a healthy aged population with attenuation of CD66b expression on neutrophils and decreased shedding of CD62L (Takahashi et al., 2013).

Contrary to previous findings in COPD, de Alencar Silva et al. (2018) recently found that an exercise programme of a similar duration to pulmonary rehabilitation induced significant reductions in circulating cytokines including IL-6. However, no studies have yet assessed whether pulmonary rehabilitation can play a role in reducing other markers of systemic inflammation, which are also widely recognised for their reliability as a surrogate marker of exacerbation risk (e.g. fibrinogen) (Duvoix et al, 2013). There is a need to further understand and investigate the effects of an exercise programme, such as pulmonary rehabilitation, on changes in resting systemic inflammation in COPD, placing emphasis on established or novel

surrogate markers of the frequent exacerbator phenotype of COPD. Should exercise be confirmed to have effects on such outcomes in COPD, it would be particularly prudent in the management of the COPD exacerbation phenotype where traits like systemic inflammation currently have limited treatment options.

The aims of this study were to examine the effects of exercise, as part of pulmonary rehabilitation, on resting markers of systemic inflammation in patients with frequent or infrequent exacerbations of COPD. It was hypothesised that exercise would reduce markers of systemic inflammation (fibrinogen & CRP) and attenuate markers of neutrophil activation but would not induce a significant decrease in resting blood leukocyte cell populations.

4.2 Methods

4.2.1 Participants

4.2.1.1 COPD patients

Forty COPD patients (age 68.8 ± 7.2 years; FEV₁ pred $49 \pm 17\%$; frequent exacerbators = 24; infrequent exacerbators = 16) volunteered to take part in this study.

4.2.1.2 Healthy comparators

Twenty-five healthy comparators (age 67.9 ± 5.5 years; FEV₁ pred $101 \pm 13\%$; smokers = 10; never smokers = 15) were recruited to provide indications of baseline comparative measures between healthy individuals and COPD patients.

4.2.2 Procedures

Blood samples were collected in a resting state at the 1st (Pre-Rehab) and 16th classes (Post-Rehab) for COPD patients. Any COPD patients reporting an inflammatory event (e.g. exacerbation) or receiving antibiotics/corticosteroids at the beginning or end of pulmonary rehabilitation were excluded from the study analyses. Single blood samples were collected from healthy comparators in a

separate clinic from COPD patients with controls put in place for time of day (between 10am and 1pm). The primary outcome was plasma fibrinogen concentration (section 2.8.2.1). Secondary outcomes were plasma CRP (section 2.8.2.2) and total and differential leukocyte count (section 2.8.1). Markers of neutrophil activation (CD11b, CD62L, CD66b) and neutrophil phenotypes (mature (CD16b^{high}/CD62L^{high}), immature (CD16b^{low}/CD62L^{high}), suppressive (CD16b^{high}/CD62L^{low}), and progenitor (CD16b^{low}/CD62L^{low})) were also determined in a subset of participants (frequent, n = 18; infrequent, n = 11; smokers = 9; never smokers = 15) whole blood samples using flow cytometry (section 2.8.3).

4.2.3 Statistical analysis

Firstly, indications of differences in baseline fibrinogen concentrations, total and differential leukocyte counts, expression of neutrophil activation markers (CD11b, CD62L, CD66b), and neutrophil maturity (mature, CD16b^{high}/CD62L^{high}, immature, CD16b^{low}/CD62L^{high}, suppressive, CD16b^{high}/CD62L^{low}, progenitor, CD16b^{low}/CD62L^{low}) were determined using a one-way ANOVA with Bonferroni correction (frequent exacerbators vs infrequent exacerbators vs healthy smokers vs healthy never smokers). Differences in CRP concentrations were analysed using a Kruskal-Wallis test. For the main analyses, changes in fibrinogen concentrations, total and differential leukocyte counts, expression of neutrophil activation markers, and neutrophil maturity were analysed pre- and post-rehabilitation in both groups (frequent vs infrequent exacerbators; 1st vs 16th) using a two-way mixed ANOVA with Bonferroni correction. Any significant main effects observed in the ANOVA were further analysed with post-hoc two-tailed paired t-tests. Changes in CRP concentrations between pre-rehabilitation and post-rehabilitation were analysed using a Wilcoxon signed rank test with differences between groups at each time

point analysed using a Mann-Whitney U test. Statistical significance was accepted at $P < 0.05$.

4.3 Results

4.3.1 Participants characteristics

Due to inflammatory events (exacerbations treated with antibiotics or corticosteroids: frequent exacerbators ($n = 8$), infrequent exacerbators ($n = 1$); sciatica: infrequent exacerbators ($n = 1$)) at the beginning or end of pulmonary rehabilitation, 10 COPD patients were removed from the analysis (frequent exacerbators, $n = 8$; infrequent exacerbators, $n = 2$). Also, plasma samples were unable to be obtained from 2 COPD patients resulting in plasma being unavailable for the analysis of fibrinogen and CRP (frequent exacerbators, $n = 1$; infrequent exacerbators, $n = 1$). In the subset of participants who were assessed for neutrophil activation markers, 18 participants were excluded from these analyses due to a lack of expression of CD16b meaning that neutrophils could not be confidently isolated for analysis (frequent exacerbators, $n = 6$; infrequent exacerbators, $n = 4$; healthy smokers, $n = 1$; healthy never smokers, $n = 7$).

The participant characteristics of frequent and infrequent exacerbators, as well as healthy smokers and healthy never smokers are detailed in Table 4.1. Further COPD specific characteristics are detailed in Table 4.2. All frequent exacerbators were categorised as GOLD grade D (100%) with the majority of infrequent exacerbators classified as GOLD grade B (93%). The majority of frequent and infrequent exacerbators were categorised as either mMRC grade 2, grade 3, or grade 4.

Table 4.1. Participant characteristics

Variable	Frequent	Infrequent	Healthy smokers	Healthy never smokers
Age (years) ^a	68.5 ± 6.8	70.6 ± 6.4	67.1 ± 3.1	68.5 ± 6.7
% Males ^b	56%	50%	50%	60%
Body Mass (kg) ^a	73.7 ± 12.6	73.3 ± 14.2	78.3 ± 24.7	77.7 ± 14.6
FEV ₁ %predicted ^a	48 ± 17	56 ± 16	93 ± 12	107 ± 11

^aData presented as mean ± SD. ^bData presented as a % of total population. Frequent, n = 16; infrequent, n = 14; healthy smokers, n = 10; healthy never smokers, n = 15.

Table 4.2. COPD specific characteristics

Variable	Frequent	Infrequent
GOLD grade, n (%) ^a		
A	0 (0)	1 (7)
B	0 (0)	13 (93)
C	0 (0)	0 (0)
D	16 (100)	0 (0)
mMRC, n (%) ^a		
0	0 (0)	1 (7)
1	0 (0)	0 (0)
2	2 (12)	4 (29)
3	7 (44)	3 (14)
4	7 (44)	7 (50)
Charlson Comorbidity Index ^b	3.8 ± 1.2	4.1 ± 1.2
Current smokers ^c	25%	21%

^aData presented as total number (% of group). ^bData presented as mean ± SD. ^cData presented as a % of total population. Frequent, n = 16; infrequent, n = 14.

4.3.2 Inflammation

4.3.2.1 Baseline observations

There was a significant main difference between groups for fibrinogen concentration ($p = 0.027$). Post-hoc analyses demonstrated that only frequent exacerbators had a significantly elevated fibrinogen concentration compared to healthy never smokers ($p = 0.026$) (Figure 4.1).

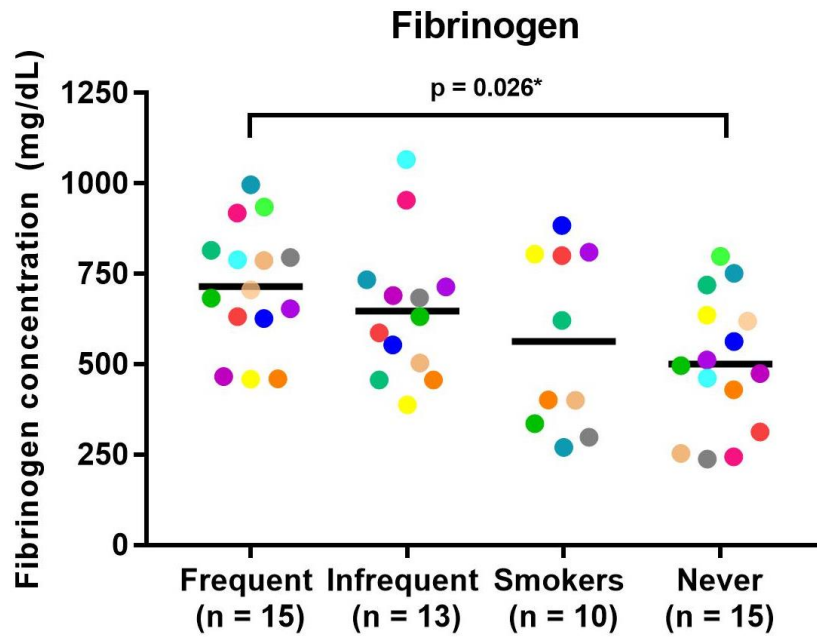


Figure 4.1. Baseline fibrinogen concentrations in frequent and infrequent exacerbators as well as healthy smokers and never smokers. Lines represent mean values. *Significant difference between groups ($p < 0.05$).

There were no significant main differences observed between groups for: CRP ($p = 0.089$), total blood leukocyte count ($p = 0.155$), neutrophil count ($p = 0.409$), eosinophil count ($p = 0.414$), lymphocyte count ($p = 0.156$), and neutrophil/lymphocyte ratio ($p = 0.384$) (Table 4.3).

Table 4.3. Baseline blood counts

Variable	Frequent	Infrequent	Healthy smokers	Healthy never smokers	p-value
CRP (mg/L) ^a	7.1 (3.5-10.8)	10.6 (2.5-11.2)	2.3 (1.4-2.2)	3.3 (2.2-8.7)	0.089
Total Leukocytes (10 ⁹ ·L ⁻¹) ^b	8.1 ± 2.0	6.9 ± 1.2	7.9 ± 2.6	6.7 ± 1.8	0.155
Neutrophils (10 ⁹ ·L ⁻¹) ^b	4.9 ± 1.6	4.1 ± 1.1	4.4 ± 1.9	4.0 ± 1.3	0.409
Eosinophils (10 ⁹ ·L ⁻¹) ^b	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.414
Lymphocytes (10 ⁹ ·L ⁻¹) ^b	2.2 ± 0.6	2.0 ± 0.5	2.5 ± 0.9	1.9 ± 0.9	0.156
Neutrophil/Lymphocytes ratio ^b	2.4 ± 1.0	2.2 ± 1.0	2.0 ± 0.8	2.4 ± 0.9	0.384

^aData presented as median (25th percentile – 75th percentile). ^bData presented as mean ± SD. CRP: frequent, n = 15; infrequent, n = 13. All other variables: frequent, n = 16; infrequent, n = 14; healthy smokers, n = 10; healthy never smokers, n = 15.

There were no significant main differences observed in the expression of CD11b between groups ($p = 0.539$). However, there was a tendency, which did not meet statistical significance, towards a main difference between groups for CD62L ($p = 0.078$) and CD66b ($p = 0.064$). There were no significant main differences between groups for the neutrophil phenotypes: mature (CD16b^{high}/CD62L^{high}, $p = 0.119$), immature (CD16b^{low}/CD62L^{high}, $p = 0.305$), and progenitor (CD16b^{low}/CD62L^{low}, $p = 0.547$). There was a tendency towards a main difference between groups for suppressive neutrophils (CD16b^{high}/CD62L^{low}, $p = 0.081$) but this was not statistically significant (Table 4.4).

Table 4.4. Baseline neutrophil activation markers

Neutrophil Marker	Frequent	Infrequent	Healthy smokers	Healthy never smokers	p-value
CD11b (MFI)	5083 ± 1157	5647 ± 1072	5644 ± 429	5474 ± 615	0.539
CD62L (MFI)	24366 ± 4093	25312 ± 7522	28958 ± 6180	31092 ± 5401	0.078
CD66b (MFI)	10326 ± 2521	9833 ± 2343	14233 ± 4561	10577 ± 2242	0.064
CD16b ^{high} /CD62L ^{high} (%)	94.2 ± 2.2	95.0 ± 2.2	94.8 ± 2.5	96.1 ± 2.9	0.119
CD16b ^{low} /CD62L ^{high} (%)	1.6 ± 0.5	2.0 ± 0.9	2.5 ± 1.9	1.5 ± 0.4	0.305
CD16b ^{high} /CD62L ^{low} (%)	4.0 ± 2.2	3.0 ± 1.6	2.6 ± 1.8	2.3 ± 2.9	0.081
CD16b ^{low} /CD62L ^{low} (%)	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.547

Data presented as mean ± SD. MFI, median fluorescent intensity. Frequent, n = 8; infrequent, n = 7; healthy smokers, n = 8; healthy never smokers, n = 8.

4.3.2.2 Responses to pulmonary rehabilitation

There was a significant main effect of time showing a reduction in plasma fibrinogen following pulmonary rehabilitation ($p = 0.033$). No significant differences were observed between frequent and infrequent exacerbators for fibrinogen ($p = 0.414$) nor were any time \times group interactions observed ($p = 0.702$) (Figure 4.2).

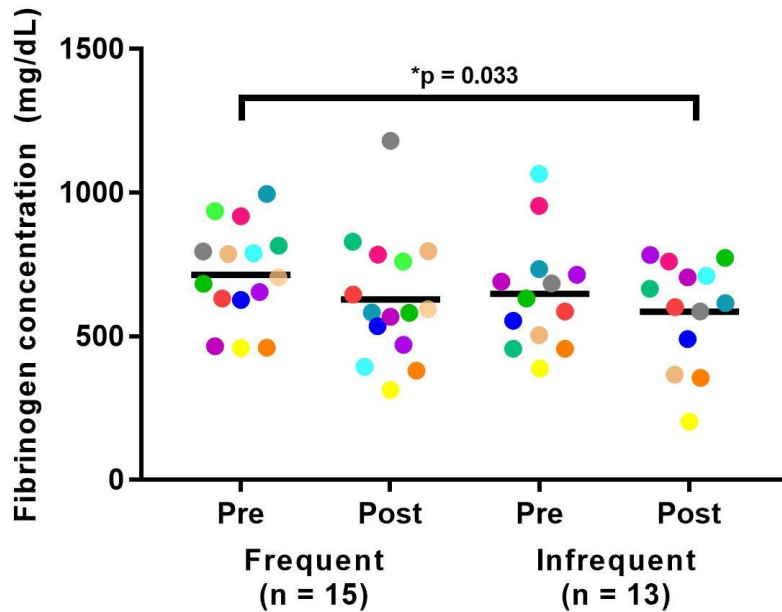


Figure 4.2. Fibrinogen concentrations in response to pulmonary rehabilitation in frequent and infrequent exacerbators. Lines represent mean values. *Significant main effect of time ($p < 0.05$).

Pulmonary rehabilitation did not significantly reduce the concentration of plasma CRP ($p = 0.733$). No significant differences for CRP concentration were observed between frequent and infrequent exacerbators at pre-rehabilitation ($p = 0.683$) and post-rehabilitation ($p = 0.586$) (Figure 4.3).

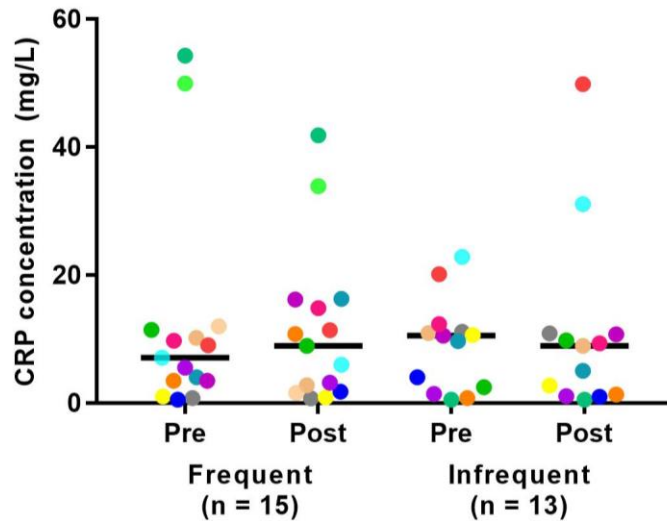


Figure 4.3. CRP concentrations in response to pulmonary rehabilitation in frequent and infrequent exacerbators. Lines represent median values.

There was a significant main effect of time on total leukocyte counts ($p = 0.032$). There was also a significant time \times group interaction with total leukocyte counts ($p = 0.041$). Post-hoc analyses showed that frequent exacerbators experienced a significant reduction in total leukocyte count by the end of pulmonary rehabilitation ($p = 0.018$), whereas infrequent exacerbators did not ($p = 0.910$). There was no significant main effect of group for total leukocyte count ($p = 0.222$) (Table 4.5).

There was a tendency towards a main effect of time on neutrophil counts, but this did not reach statistical significance ($p = 0.057$). However, there was a significant time \times group interaction with neutrophil counts ($p = 0.036$). Post-hoc analyses showed that frequent exacerbators experienced a significant reduction in neutrophil count by the end of pulmonary rehabilitation ($p = 0.018$), whereas infrequent exacerbators did not ($p = 0.849$). There was no significant main effect of group for neutrophil count ($p = 0.485$) (Table 4.5).

No effects of time (lymphocytes, $p = 0.115$; eosinophils, $p = 0.723$; neutrophil/lymphocyte, $p = 0.524$), group (lymphocytes, $p = 0.454$; eosinophils, $p = 0.479$; neutrophil/lymphocyte, $p = 0.916$), or time \times group interactions (lymphocytes,

p = 0.570; eosinophils, p = 0.846; neutrophil/lymphocyte, p = 0.123) were observed for lymphocytes, eosinophils, and neutrophil/lymphocyte ratio (Table 4.5).

Table 4.5. Blood cell counts pre- and post-pulmonary rehabilitation

Variable	Frequent	Infrequent	p-values		
			Time main effect	Time x group interaction	Group main effect
Total leukocytes (10 ⁹ ·L ⁻¹)			0.032*	0.041 [#]	0.222
Pre-Rehab	8.1 ± 2.0	6.9 ± 1.2			
Post-Rehab	6.7 ± 1.1	6.9 ± 1.2			
Neutrophils (10 ⁹ ·L ⁻¹)			0.057	0.036 [#]	0.485
Pre-Rehab	4.9 ± 1.0	4.1 ± 1.1			
Post-Rehab	3.8 ± 1.0	4.1 ± 1.1			
Eosinophils (10 ⁹ ·L ⁻¹)			0.723	0.846	0.479
Pre-Rehab	0.2 ± 0.1	0.2 ± 0.1			
Post-Rehab	0.2 ± 0.1	0.2 ± 0.1			
Lymphocytes (10 ⁹ ·L ⁻¹)			0.115	0.570	0.454
Pre-Rehab	2.2 ± 0.6	2.0 ± 0.4			
Post-Rehab	2.0 ± 0.5	1.9 ± 0.4			
Neutrophil/Lymphocyte ratio			0.524	0.123	0.916
Pre-Rehab	2.4 ± 1.0	2.2 ± 1.0			
Post-Rehab	2.1 ± 0.8	2.3 ± 0.8			

Data presented as mean ± SD. *Significant main effect of time (p < 0.05); [#]Significant time x group interaction (p < 0.05). Frequent, n = 16; infrequent, n = 14.

Pulmonary rehabilitation did not appear to significantly alter the cell surface expression of CD11b ($p = 0.553$) or CD66b on blood neutrophils ($p = 0.317$). However, there was a tendency towards an increase in the expression of CD62L by the end of pulmonary rehabilitation, but this was not found to be statistically significant ($p = 0.070$). There was no significant main effect of group in blood neutrophil expression of CD11b ($p = 0.161$), CD62L ($p = 0.503$), or CD66b ($p = 0.906$). Additionally, there were no significant time \times group interactions (CD11b, $p = 0.936$; CD62L, $p = 0.491$; $p = 0.168$) (Table 4.6).

Table 4.6. Neutrophil surface expression pre- and post-pulmonary rehabilitation

			p-values		
			Time		Group
Neutrophil			main	Time \times group	main
Marker	Frequent	Infrequent	effect	interaction	effect
CD11b (MFI)			0.553	0.936	0.161
Pre-Rehab	5083 \pm 1157	5647 \pm 1072			
Post-Rehab	5294 \pm 667	5809 \pm 647			
CD62L (MFI)			0.070	0.491	0.503
Pre-Rehab	24366 \pm 4093	25312 \pm 7522			
Post-Rehab	26036 \pm 4797	28209 \pm 2087			
CD66b (MFI)			0.317	0.168	0.906
Pre-Rehab	10326 \pm 2521	9833 \pm 2343			
Post-Rehab	10064 \pm 1979	10735 \pm 2043			

Data presented as mean \pm SD. MFI, median fluorescent intensity. Frequent, $n = 8$; infrequent, $n = 7$.

A significant main effect of time was observed with reductions in the progenitor neutrophil subset by the end of pulmonary rehabilitation ($p = 0.015$). There were no main effects of time observed with any of the other neutrophil subsets (mature, $p = 0.313$; immature, $p = 0.756$; suppressive, $p = 0.259$). There was no main effect of group (mature, $p = 0.517$; immature, $p = 0.172$; suppressive, $p = 0.261$; progenitor, $p = 0.999$) or time \times group interactions observed with any of the neutrophil subsets (mature, $p = 0.805$; immature, $p = 0.780$; suppressive, $p = 0.809$; progenitor, $p = 0.193$) (Table 4.7).

Table 4.7. Neutrophil phenotypes pre- and post-pulmonary rehabilitation

Neutrophil Phenotype	Frequent	Infrequent	p-values		
			Time main effect	Time \times group interaction	Group main effect
CD16b ^{high} /CD62L ^{high} (%)			0.313	0.805	0.517
Pre-Rehab	94.2 \pm 2.2	95.0 \pm 2.1			
Post-Rehab	94.8 \pm 2.3	95.3 \pm 1.3			
CD16b ^{low} /CD62L ^{high} (%)			0.756	0.780	0.172
Pre-Rehab	1.6 \pm 0.5	2.0 \pm 0.9			
Post-Rehab	1.5 \pm 0.6	2.0 \pm 0.9			
CD16b ^{high} /CD62L ^{low} (%)			0.259	0.809	0.261
Pre-Rehab	4.0 \pm 2.2	3.0 \pm 1.6			
Post-Rehab	3.7 \pm 2.2	2.6 \pm 1.3			
CD16b ^{low} /CD62L ^{low} (%)			0.015*	0.193	0.999
Pre-Rehab	0.2 \pm 0.2	0.1 \pm 0.1			
Post-Rehab	0.1 \pm 0.1	0.1 \pm 0.0			

Data presented as mean \pm SD. *Significant main effect of time ($p < 0.05$). Frequent, $n = 8$; infrequent, $n = 7$.

4.4 Discussion

The main findings from this study suggest that pulmonary rehabilitation can reduce circulating fibrinogen concentrations and the percentage of progenitor neutrophils. This study also found that pulmonary rehabilitation is capable of reducing total blood leukocyte and neutrophil counts in frequent exacerbators of COPD. Pulmonary rehabilitation did not significantly reduce the concentration of CRP or modify markers of neutrophil activation in the frequent or infrequent exacerbator phenotypes of COPD.

Fibrinogen is an established biomarker for exacerbations of COPD (Chen, Leung & Sin, 2016). In the context of pulmonary rehabilitation, this is the first study in the COPD population to demonstrate that pulmonary rehabilitation can be effective in modulating resting concentrations of fibrinogen irrespective of exacerbation history. This evidence agrees with that of previous studies in a healthy population demonstrating that an exercise programme can be effective in reducing plasma fibrinogen (Ernst, 1993; Dehghan & Faramarzi, 2013; Gomez-Marcos et al., 2014; Rashidlamir & Jaafari, 2013). Pulmonary rehabilitation has also been seen to reduce fibrinogen levels in patients with lung cancer (Morano et al., 2014). Others, however, have not observed any significant reductions in fibrinogen concentrations following a period of exercise training in healthy individuals and patients with peripheral obstructive arterial disease (Clark et al., 2011; Furukawa et al., 2008; Hammett et al., 2006; Mika et al., 2011). Fibrinogen has been demonstrated to have pro-inflammatory properties via stimulating the production of MMP-9 and inhibiting anti-inflammatory cytokines (Wang et al., 2015). Reductions in this marker can be interpreted as an anti-inflammatory response to exercise. It is plausible to suggest that this reduction in fibrinogen concentration may provide mechanistic evidence towards a reduction in exacerbation risk following pulmonary rehabilitation in COPD

(Moore et al., 2016). However, this requires further research with more longitudinal based studies assessing fibrinogen, exercise and exacerbation reporting.

CRP is an acute phase protein indicative of inflammation and a marker that has been established as a biomarker for COPD exacerbations (Chen, Leung & Sin, 2016). This study suggests that pulmonary rehabilitation is not effective at modifying resting levels of CRP. This is in agreement with previous work in COPD populations (El Gammal et al., 2015; Sciriha et al., 2017; Vogiatzis et al., 2007). The findings also agree with previous research in a healthy sedentary population displaying elevated CRP levels, whereby 4 months of exercise training did not decrease CRP concentrations (Church et al., 2010). However, CRP was highly variable between patients in the present study, which is commonly seen in the COPD population (Aksu et al., 2013; Silva, Gazzana & Knorst, 2015), perhaps suggesting a larger sample size would be required to detect any significant differences. The degree of intra-variation with CRP suggests it would be challenging to assess responses of CRP to exercise in COPD.

Total blood leukocyte and neutrophil counts have also been shown to be significantly elevated in frequent exacerbators (Hurst et al., 2010). This is the first study to show that pulmonary rehabilitation can significantly reduce total blood leukocyte and neutrophil counts in frequent exacerbators. This conflicts with previous work in a COPD population where pulmonary rehabilitation has not been shown to significantly reduce total blood leukocyte or neutrophil counts (El Gammal et al., 2015; Sciriha et al., 2017). These studies have assessed responses in the COPD population as a whole, whereas the current study stratified patients by exacerbation history. The findings suggest that in clinical phenotypes where there is scope for anti-inflammatory benefits (e.g. frequent exacerbators of COPD), total blood leukocyte or neutrophil counts are amenable to change by pulmonary

rehabilitation. However, further research is required assessing the inflammatory cell count responses to exercise in both frequent and infrequent exacerbators to further support these findings.

It has previously been proposed that neutrophil counts alone provide little insight into inflammatory responses, whereby assessing markers of neutrophil activation markers may be more worthwhile (Oudijk et al., 2005). This is the first study in COPD to assess neutrophil activation markers in the context of pulmonary rehabilitation. This study did not find any significant changes in neutrophil activation markers (CD11b, CD62L and CD66b) following pulmonary rehabilitation. However, tendencies towards increases observed with CD62L expression by the end of pulmonary rehabilitation suggest that this is a promising activation marker for future research assessing changes with chronic exercise. This is suggested to be indicative of an anti-inflammatory effect of exercise training as shedding of CD62L is observed upon neutrophil activation (Borregaard & Cowland, 1997; Ley et al., 2007; Nathan, 2006; Takahashi et al., 2013; Wittmann et al., 2004). Such findings are in agreement with the findings of Takahashi et al. (2013) who found that exercise training in an aged population increased the resting expression of CD62L indicating an anti-inflammatory response to exercise training. Power calculations suggested that 75 COPD patients would be required to detect statistical significance at 80% power and 5% level of significance in neutrophil CD62L expression with a change of 2243 MFI. The current study however, did agree with research by Nawaz et al. (2001) who examined CD66b and CD11b neutrophil expression following an exercise training programme in patients with intermittent claudication where no effects were observed. However, further investigations of these markers are warranted given the significance of CD11b and CD66b in neutrophil activation in COPD (Fortunati et al., 2009).

For the first time in an exercise and COPD setting, phenotyping of blood neutrophils (Cortjens et al., 2017), was performed in this study. This study identified that pulmonary rehabilitation can reduce the amount of progenitor neutrophils in COPD. The progenitor subset is a newly identified small subset of neutrophils, considered to have increased degranulation (Cortjens et al., 2017). Progenitor neutrophils have also been suggested to have a poorer migration due to reduced CD62L expression (Cortjens et al., 2017). Therefore, a reduction in this subset could be deemed as a beneficial effect of exercise with a shift towards neutrophil subsets with enhanced migration and reduced degranulation (Cortjens et al., 2017). However, no significant differences were observed in any of the other neutrophil subsets at the end of pulmonary rehabilitation, so whether this decrease results in more mature, immature or suppressive neutrophils is unclear. These findings were based on observations made in a limited sample size with further research warranted assessing shifts in neutrophil subsets in both frequent and infrequent exacerbators of COPD in response to exercise to determine pro- and anti-inflammatory effects.

The findings from the current study imply that pulmonary rehabilitation can have anti-inflammatory effects in frequent and infrequent exacerbators. However, not all inflammatory parameters were affected which may be indicative of an issue with the duration, frequency and/or intensity of the exercise programme (Kruger & Mooren, 2007; McCarthy & Dale, 1988; Reihmane et al., 2013). Previously it has been indicated that markers such as total leukocyte and neutrophil counts are more sensitive to acute inflammatory responses to exercise (van Helvoort et al., 2006). Therefore, future research should assess how these markers respond to acute exercise and whether modification of the acute inflammatory response changes across pulmonary rehabilitation, as seen in exercise training studies in healthy populations (Brown et al., 2015). Acute inflammatory responses to exercise may

bear more relevance to patients with COPD given the inflammatory nature of the disease, whereby acute bursts of inflammation could be deleterious and increase the risk of exacerbation (van der Vlist & Janssen, 2010).

The findings presented require further exploration but begin to assess previously unexplored mechanisms of exercise interventions and incidence of exacerbations of COPD (Jenkins et al., 2018; Jenkins, Holden & Jones, 2018; Moore et al., 2016). Further research establishing the anti-inflammatory benefits of exercise and pulmonary rehabilitation, specifically biomarkers relating to exacerbations, in COPD may provide a major selling point for the intervention which is currently underfunded and underutilised (Hoogendoorn et al., 2010; Jones et al., 2014b; Jones et al., 2017; Keating, Lee, & Holland, 2011; Marciniuk et al., 2010; Mayers et al., 2007; Oba, 2007).

When interpreting the results of this study it is important to acknowledge limiting factors. The comparisons of baseline inflammatory markers were not part of the main analyses and utilised small sample sizes between frequent exacerbators, infrequent exacerbators, healthy smokers, and healthy never smokers so the findings were underpowered. The pulmonary rehabilitation course provided was a conventional course delivered in accordance with BTS guidelines (Bolton et al., 2013). Therefore, no strict restrictions were put in place to standardise the exercise training across participants but exercise workload was continually progressed throughout the course according to physical capabilities of each patient in line with usual care procedures. Whilst healthy comparator groups were recruited to demonstrate the altered markers of systemic inflammation at rest in COPD exacerbation phenotypes, this study did not recruit a control group of COPD over the course of pulmonary rehabilitation. Hence the observed effects over time cannot be specifically attributed to the effect of pulmonary rehabilitation alone. The main

aim of this study was to compare responses between frequent and infrequent exacerbators but it would be prudent for future studies to include such patient groups that are not exposed to pulmonary rehabilitation to better understand the magnitude of effect on inflammation. It is also important to note that the final analysis of neutrophil activation markers was conducted on a small sample size and future larger-scale research is required to confirm the current findings. This small sample size was attributed to unforeseen issues with using CD16b as a marker for identifying neutrophil populations whereby 43% of frequent exacerbators and 36% of infrequent exacerbators did not highly express CD16b on neutrophils. This is an unusual phenomenon and very little literature is available to explain this. One suggestion raised in the literature is a very rare genetic deficiency of CD16 (Wagner & Hansch, 2004), with another suggestion that ADAM17, which is elevated in COPD (Stolarczyk & Scholte, 2018), cleaves neutrophil CD16b (Wang et al., 2013), but assessment of CD16 genes and ADAM17 were not undertaken in the current study. However, this neutrophil CD16b deficiency was not observed to be COPD specific as similar observations were made in 11% of healthy smokers and 47% of healthy never smokers. Due to this deficiency in CD16b, the confidence in being able to identify the neutrophil population was reduced, so these participants were not included in the analysis meaning the results were underpowered. This would suggest that CD16b is not the most suitable marker for identifying neutrophil populations. Smokers were also included in the present study, so interpretations about findings should be undertaken with caution as smoking has been shown to enhance inflammation (Lee, Taneja & Vassallo, 2012). This study also excluded COPD patients with other co-morbid respiratory conditions in order to ensure exacerbations were related to COPD and not that of other respiratory conditions. However, a strength of the study was that the findings reflect 'real-world' effects in

COPD patients presenting without other co-morbid respiratory conditions, whereby pulmonary rehabilitation was not manipulated in any way by the study.

In conclusion, pulmonary rehabilitation appears to have positive effects in reducing plasma fibrinogen concentrations in COPD and may provide more anti-inflammatory benefits to frequent exacerbators as characterised by reduced total blood leukocyte and neutrophil counts. Further research is required to assess chronic immune responses to pulmonary rehabilitation utilising a wider range of biomarkers for exacerbations.

5 Chapter FIVE

Study 3 – The effects of pulmonary rehabilitation on corticosteroid-inducible gene expression in PBMC's of frequent and infrequent exacerbators of COPD

Abstract

Corticosteroids are a commonly used pharmacological treatment of COPD. ICS are often prescribed as an anti-inflammatory therapy in frequent exacerbators. However, COPD patients have been seen to be resistant to the effects of ICS calling into question the effectiveness of corticosteroids. The aim of this study was to investigate the effects of pulmonary rehabilitation on the expression of corticosteroid-induced anti-inflammatory genes. PBMC's were isolated from whole blood samples of 7 frequent (FEV_1 pred, $46 \pm 17\%$) and 7 infrequent exacerbators (FEV_1 pred, $61 \pm 14\%$) pre- and post-rehabilitation. These were compared to PBMC's isolated from 8 healthy smokers (FEV_1 pred, $95 \pm 13\%$) and 8 healthy never smokers (FEV_1 pred, $107 \pm 14\%$). PBMC's were treated with corticosteroids for 2 & 6 hours before messenger RNA (mRNA) was extracted for the analysis of MKP-1 & GILZ gene expression via real-time PCR. Frequent exacerbators had a similar response in MKP-1 expression to dexamethasone treatment compared to infrequent exacerbators ($p = 0.272$), healthy smokers ($p = 0.098$) and healthy never smokers ($p < 0.999$). Pulmonary rehabilitation showed a tendency towards an increased MKP-1 expression in both frequent and infrequent exacerbators with 2 hr treatment ($p = 0.060$). Frequent exacerbators had a poorer fold increase in MKP-1 expression following 6 hr treatment with dexamethasone at both pre- and post-rehabilitation compared to infrequent exacerbators ($p = 0.003$). GILZ expression was not significantly influenced by pulmonary rehabilitation (2 hr, $p = 0.774$; 6 hr, $p = 0.734$). This study suggests that pulmonary rehabilitation may play a role in modulating the effectiveness of corticosteroids in both frequent and infrequent

exacerbators of COPD. Whether these potential increases in anti-inflammatory gene mRNA results in increased clinical effectiveness of corticosteroids is still to be elucidated.

5.1 Introduction

Large sums of money are spent worldwide on pharmacological therapies to help reduce the burden of COPD (World Health Statistics, 2011). In COPD, the effectiveness of pharmacological therapies, for example, ICS have been called into question (Ernst, Saad & Suissa, 2015). The proposed use of ICS in COPD has been largely due to their anti-inflammatory properties (Jen, Rennard & Sin, 2012), and history of benefit in respiratory diseases such as asthma (Barnes, 2010). It has been suggested that ICS reduce exacerbation frequency and lead to improvements in health-related quality of life (Spencer et al., 2004; Suissa et al., 2007; Wedzicha, Mackay & Singh, 2013). However, some patients with COPD have been seen to develop insensitivity to corticosteroids (Lo Tam Loi et al., 2013), with long-term use also associated with an increased risk of pneumonia (Suissa et al., 2013).

There have been attempts to provide a pathological understanding of the reduced effectiveness of corticosteroids in COPD (Barnes, 2000; Wilkie, Finch & Schembri, 2015). It is believed that corticosteroids are more effective in situations where inflammation is underpinned by eosinophilia and research suggests that corticosteroids are not as effective in situations of neutrophilic inflammation (Kelly et al., 2012; Pascoe et al., 2015; Pavord et al., 2016). Neutrophilic inflammation is apparent in viral exacerbations and in a large proportion of COPD patients (Hoenderdos & Condliffe, 2013; Mallia et al., 2011), which may in part explain the reduced effectiveness of corticosteroids. However, there is still a dependency on the use of corticosteroids in COPD patients (NICE, 2018), especially in frequent exacerbators who have been observed to be more likely to be treated with corticosteroids (Capozzolo et al., 2017). Despite this, the same study found that patients categorised as having severe COPD will still sustain frequent exacerbations regardless of aggressive therapy maintenance (Capozzolo et al.,

2017). New recommendations suggest that ICS only be used in combination with LABA treatment in frequent exacerbators, and if blood eosinophil counts exceed 100 cells/ μ l (GOLD, 2019). This suggestion is based on evidence showing that the effects of ICS are more pronounced in patients with greater exacerbation frequency (Lipson et al., 2018).

The effectiveness of corticosteroids can be measured by examining changes in gene expression. Previously many of the anti-inflammatory effects of corticosteroids have been believed to be because of transrepression of pro-inflammatory transcription factors, but there is considerable growing evidence for the importance of corticosteroid-dependent transactivation of anti-inflammatory genes for reducing inflammation (Newton & Holden, 2007). Genes, directly induced by corticosteroids, have been established previously (Kelly et al., 2012; King et al., 2009; Newton & Holden, 2007). MKP-1 is believed to play a role in inhibiting the pro-inflammatory response (Barnes, 2008). These anti-inflammatory actions are due to the ability of MKP-1 to inactivate pro-inflammatory MAPK's, in particular p38 and extracellular signal-regulated kinases (ERK), which results in decreased cytokine production (Barnes, 2008). GILZ is also an established corticosteroid-inducible gene and targets key inflammatory transcription factors suppressing inflammatory gene expression (Berrebi et al., 2003; Eddleston et al., 2007; Godot et al., 2006; Mittelstadt & Ashwell, 2001). Therefore, cells with a reduced expression of these corticosteroid-inducible genes have been seen to have an impaired response to corticosteroids (Kang et al., 2008; Lasa et al., 2002).

There have been calls for a better understanding of corticosteroids in COPD to improve treatment response (Leung & Sin, 2018). It has been previously suggested that non-pharmacological therapies such as exercise can provide similar benefits to that of pharmacological therapies in terms of disease prevention and control

(Vina et al., 2012). However, whether exercise can play a role in enhancing the effectiveness of pharmacological therapies in COPD is unclear. In Study 2, we established the capacity of pulmonary rehabilitation to reduce systemic inflammation (i.e. pro-inflammatory factors) but whether programmes can also impact on the action of anti-inflammatory treatments in the form of corticosteroids remains unclear. In COPD, it has been suggested that patients may need pulmonary rehabilitation to fully achieve the benefits of current and new therapies (Katajisto et al., 2015). Current suggestions are that the benefits of pulmonary rehabilitation on pharmacological treatment are indirect, such as a result of increased knowledge and/or better inhaler technique as opposed to any direct biological effects (Evans & Steiner, 2018). However, these suggestions are largely hypothetical and hence there remains a lack of research that have explored the underlying mechanisms.

Therefore, the aim of this study was to assess whether pulmonary rehabilitation could increase the expression of corticosteroid inducible genes (MKP-1 & GILZ) in frequent and/or infrequent exacerbators of COPD. It was hypothesised that corticosteroid inducible gene expression would be lower in frequent exacerbators, but that pulmonary rehabilitation would increase the effectiveness of corticosteroids in both frequent and infrequent exacerbators.

5.2 Methods

5.2.1 Participants

5.2.1.1 COPD patients

Seven frequent (age 67 ± 7 years; FEV₁ pred $46 \pm 17\%$) and 7 infrequent exacerbators (age 68 ± 7 years; FEV₁ pred $61 \pm 14\%$) took part in this study based on sample sizes previously utilised in intervention studies assessing changes in MKP-1 & GILZ gene expression (Kelly et al., 2012; King et al., 2009).

5.2.1.2 Healthy comparators

Eight smokers (age 68 ± 3 years; FEV₁ pred $95 \pm 13\%$) and 8 healthy never smokers (age 69 ± 8 years; FEV₁ pred $107 \pm 14\%$) were also recruited to provide reference control groups (baseline comparisons) in this study.

5.2.2 Procedures

Blood samples were collected in a resting state at the 1st (Pre-Rehab) and 16th classes (Post-Rehab) of pulmonary rehabilitation for the COPD patients. Single resting blood samples were collected at a similar time of day (between 10am and 1pm) for the healthy comparators in separate clinics from the COPD patients. PBMC's were isolated using a density medium and treated with dexamethasone (1 μ M) (not-treated (NT), 2 hr, 6 hr) as outlined in section 2.8.4. The expression of corticosteroid inducible genes MKP-1 and GILZ were measured by real-time PCR (section 2.8.4).

The primary aim of this study was to assess changes in corticosteroid-inducible gene expression (MKP-1 & GILZ) with pulmonary rehabilitation. The secondary aims of this study were to assess changes in gene expression following differing treatments (2 hr & 6 hr) and compare gene expression between groups (frequent exacerbators, infrequent exacerbators, healthy smokers, healthy never smokers).

5.2.3 Statistical analyses

Comparisons of NT raw data were analysed across all groups using a Kruskal-Wallis test. Changes in NT samples pre- to post-pulmonary rehabilitation were compared within groups (frequent and infrequent exacerbators) using paired samples t-tests. An independent t-test was utilised to compare NT raw data post-rehab between frequent and infrequent exacerbators. Comparisons of dexamethasone treated samples (2 hr & 6 hr) for each gene (MKP-1 & GILZ) were analysed as fold increases against NT samples (normalised to 1) between all

groups (frequent exacerbators, infrequent exacerbators, healthy smokers, healthy never smokers) using a two-way mixed ANOVA with Bonferroni correction. Changes in gene expression, expressed as a fold against NT, at 2 hr and 6 hr treatments (MKP-1 & GILZ) were analysed pre- and post-rehabilitation in frequent and infrequent exacerbators using a two-way mixed ANOVA with Bonferroni correction. Any significant main effects were further analysed with post-hoc paired t-tests. Statistical significance was accepted at $P < 0.05$.

5.3 Results

5.3.1 Participant characteristics

The participant characteristics of frequent and infrequent exacerbators, as well as healthy smokers and healthy never smokers are detailed in Table 5.1. Further COPD specific characteristics are detailed in Table 5.2. All frequent exacerbators were categorised as GOLD grade D (100%) with the majority of infrequent exacerbators classified as GOLD grade B (86%). The majority of frequent and infrequent exacerbators were categorised as either mMRC grade 2, grade 3, or grade 4.

Table 5.1. Participant characteristics

Variable	COPD		HEALTHY	
	Frequent	Infrequent	Smokers	Never smokers
Age (years) ^a	67.4 ± 7.3	68.0 ± 7.0	67.5 ± 3.3	69.1 ± 7.8
% Males ^b	71%	57%	50%	75%
Body Mass (kg) ^a	75.0 ± 14.5	76.9 ± 5.9	80.3 ± 22.3	74.8 ± 11.2
FEV ₁ %predicted ^a	46 ± 17	61 ± 14	95 ± 13	107 ± 14

^aData presented as mean ± SD. ^bData presented as a % of total population. Frequent, n = 7; infrequent, n = 7; smokers, n = 8; never smokers = 8.

Table 5.2. COPD specific characteristics

Variable	Frequent	Infrequent
GOLD grade, n (%) ^c		
A	0 (0)	1 (14)
B	0 (0)	6 (86)
C	0 (0)	0 (0)
D	7 (100)	0 (0)
Charlson Comorbidity Index ^a	4.0 ± 1.7	3.4 ± 1.0
Current smokers ^b	17%	40%
mMRC, n (%) ^c		
0	0 (0)	1 (14)
1	0 (0)	0 (0)
2	2 (29)	1 (14)
3	2 (29)	2 (29)
4	3 (42)	3 (43)
ICS users, n (%) ^c	5 (71)	4 (57)
Daily beclomethasone equivalent (µg) ^a	1120 ± 502	1100 ± 600

^aData presented as mean ± SD. ^bData presented as a % of total population. ^cData presented as total number (% of group). Frequent, n = 7; infrequent, n = 7.

5.3.2 Gene expression

5.3.2.1 COPD patients & healthy comparators

5.3.2.1.1 Gene expression in not-treated samples

A significant main difference between groups for MKP-1 mRNA expression in PBMC's was observed for NT samples ($p = 0.006$). Post-hoc tests showed that infrequent exacerbators had a lower basal MKP-1 expression in NT PBMC's when compared to frequent exacerbators ($p = 0.019$) and healthy never smokers ($p = 0.010$), but similar expression when compared to healthy smokers ($p = 0.341$). Frequent exacerbators were observed to have ~3x and healthy never smokers ~3.5x higher MKP-1 mRNA expression in NT PBMC's compared to infrequent exacerbators. Post-hoc analyses demonstrated no significant differences between frequent exacerbators, healthy smokers, and healthy never smokers (all comparisons, $p < 0.999$).

A significant main difference between groups for GILZ mRNA expression in PBMC's was observed for NT samples ($p = 0.005$). Post-hoc tests showed that infrequent exacerbators had a lower GILZ expression in NT PBMC's when compared to healthy never smokers ($p = 0.008$), and healthy smokers ($p = 0.020$). Infrequent exacerbators had a tendency towards a lower basal GILZ expression compared to frequent exacerbators but this was not statistically significant ($p = 0.055$). Frequent exacerbators and healthy smokers were observed to have ~3.5x higher, and healthy never smokers ~2.5x higher GILZ mRNA expression in NT PBMC's compared to infrequent exacerbators. No significant differences were observed between frequent exacerbators, healthy smokers, and healthy never smokers (all comparisons, $p < 0.999$).

5.3.2.1.2 Fold changes following dexamethasone treatment

There was a significant main effect of time showing an increased expression of MKP-1 following treatment with dexamethasone ($p < 0.001$). A significant time \times group interaction was observed for MKP-1 expression ($p < 0.001$). Post-hoc t-tests demonstrated increases in MKP-1 expression with corticosteroid treatment in frequent (NT vs 2 hr, $p = 0.001$; NT vs 6 hr, $p < 0.001$) and infrequent exacerbators (NT vs 2 hr, $p = 0.001$; NT vs 6 hr, $p < 0.001$; 2 hr vs 6 hr, $p = 0.005$). No significant differences for MKP-1 expression were observed in frequent exacerbators between 2 hr and 6 hr treatments ($p = 0.258$). Increases in MKP-1 expression between NT vs 2 hr (healthy smokers, $p < 0.001$; healthy never smokers, $p = 0.001$) and NT vs 6 hr (healthy smokers, $p < 0.001$; healthy never smokers, $p = 0.001$) were observed in the healthy groups, but no significant increases were observed between 2 hr vs 6 hr (healthy smokers, $p = 0.573$; healthy never smokers, $p = 0.064$). For frequent exacerbators, fold increases from NT of $\sim 3.5x$ (2 hr time point) and $\sim 3.5x$ (6 hr time point) were observed. For infrequent exacerbators, fold increases from NT of $\sim 5x$ (2 hr time point) and $\sim 8x$ (6 hr time point) were observed. For healthy smokers, fold increases of $\sim 7.5x$ (2 hr time point) and $\sim 6x$ (6 hr time point) were observed. Finally, healthy never smokers had fold increases from NT of $\sim 4.5x$ (2 hr time point) and $\sim 3x$ (6 hr timepoint). A significant main difference between groups was observed for MKP-1 expression ($p = 0.009$). Post-hoc tests showed that healthy smokers were observed to have an enhanced MKP-1 response to dexamethasone treatment compared to healthy never smokers ($p = 0.027$). No differences were observed between frequent exacerbators vs infrequent exacerbators ($p = 0.272$), frequent exacerbators vs healthy smokers ($p = 0.098$), frequent exacerbators vs healthy never smokers ($p < 0.999$), infrequent exacerbators vs healthy smokers ($p < 0.999$),

and infrequent exacerbators vs healthy never smokers ($p = 0.088$) for MKP-1 expression following treatment with dexamethasone (Figure 5.1).

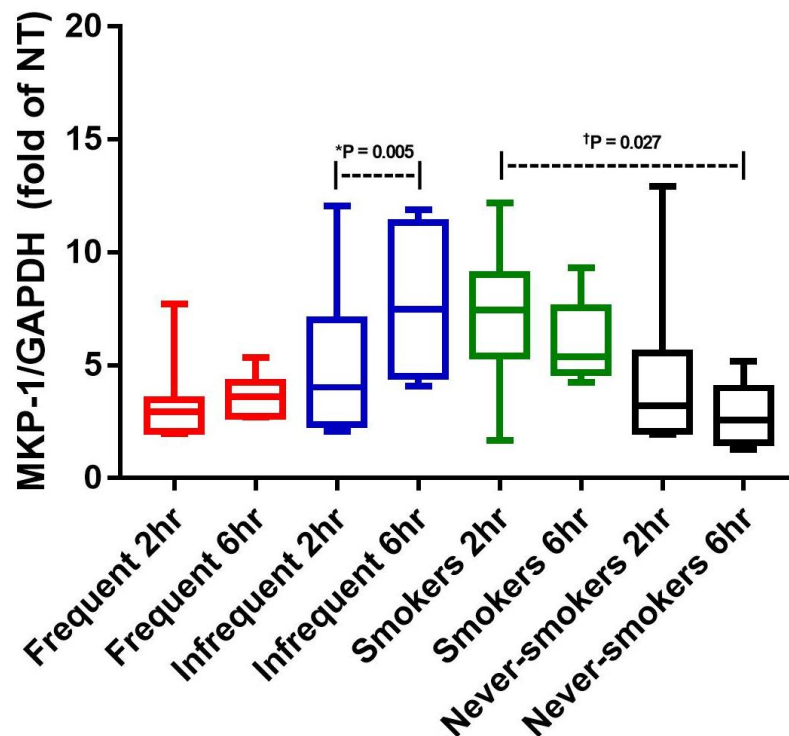


Figure 5.1. Fold changes in MKP-1 gene expression from NT in isolated PBMC's following 2 hr or 6 hr of 1 μ M dexamethasone treatment in frequent and infrequent exacerbators of COPD compared to healthy smokers and never smokers. *Significant increase between time points ($p < 0.05$). †Significant difference between groups ($p < 0.05$).

There was a significant main effect of time showing an increased expression of GILZ following treatment with dexamethasone (main effect, $p < 0.001$; NT vs 2 hr, $p = 0.001$; NT vs 6 hr, $p = 0.001$). No significant main effect was observed between 2 hr vs 6 hr ($p < 0.999$). For frequent exacerbators, fold increases from NT of ~6.5x (2 hr time point) and ~7x (6 hr time point) were observed. For infrequent exacerbators, fold increases from NT of ~12.5x (2 hr time point) and ~14x (6 hr time point) were observed. For healthy smokers, fold increases of ~11x (2 hr time point) and ~8.5x (6 hr time point) were observed. Finally, healthy never smokers had fold increases from NT of ~10.5x (2 hr time point) and ~9x (6 hr timepoint). No significant time \times group interaction was observed for GILZ expression ($p = 0.176$). There were

no significant main differences between groups for GILZ expression ($p = 0.227$) (Figure 5.2).

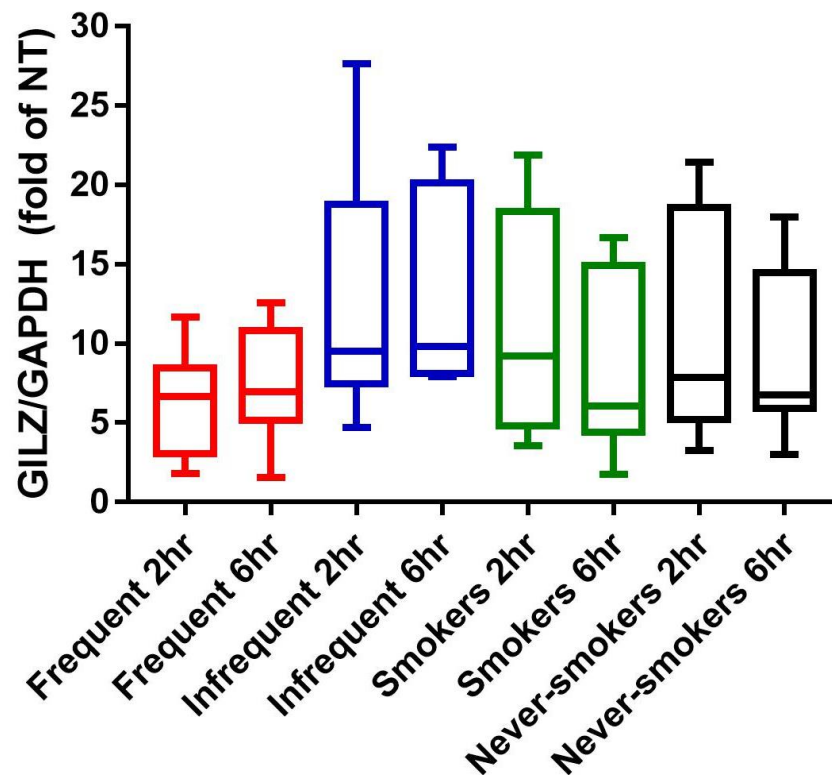


Figure 5.2. Fold changes in GILZ gene expression from NT in isolated PBMC's following 2 hr or 6 hr of 1 μ M dexamethasone treatment in frequent and infrequent exacerbators of COPD compared to healthy smokers and never smokers.

5.3.2.2 Responses to pulmonary rehabilitation

5.3.2.2.1 Gene expression in not-treated samples

Following completion of pulmonary rehabilitation, no significant differences in MKP-1 mRNA expression were observed in NT samples of frequent exacerbators when compared to pre-rehabilitation ($p = 0.134$). Similarly, no significant differences in NT MKP-1 expression were observed between pre- and post-pulmonary rehabilitation in infrequent exacerbators ($p = 0.279$). No significant differences were observed between frequent and infrequent exacerbators for MKP-1 expression in NT samples at post-rehabilitation ($p = 0.265$).

No significant differences in GILZ mRNA expression were observed in NT samples of frequent exacerbators when compared to pre-rehabilitation ($p = 0.873$). There was a significant increase in basal GILZ expression post-rehabilitation in infrequent exacerbators ($p = 0.019$). This change in basal GILZ expression in infrequent exacerbators was equivalent to a ~3.5x fold increase. No significant differences in GILZ expression of NT samples were observed between groups post-rehabilitation ($p = 0.624$).

5.3.2.2.2 Fold changes following dexamethasone treatment

Pulmonary rehabilitation showed a tendency towards an increased MKP-1 gene expression with 2 hr of dexamethasone treatment in both groups, but this was not found to be statistically significant ($p = 0.060$). No significant time \times group interactions ($p = 0.719$) or differences between groups ($p = 0.250$) were observed with MKP-1 gene expression following 2 hr treatment with dexamethasone (Figure 5.3A).

No main effect of time with pulmonary rehabilitation was observed for GILZ expression following 2 hr treatment with dexamethasone ($p = 0.774$). No significant main time \times group interactions ($p = 0.192$) or differences between groups ($p = 0.167$) were observed for GILZ gene expression following 2 hr treatment with dexamethasone at pre-rehabilitation and post-rehabilitation (Figure 5.3B).

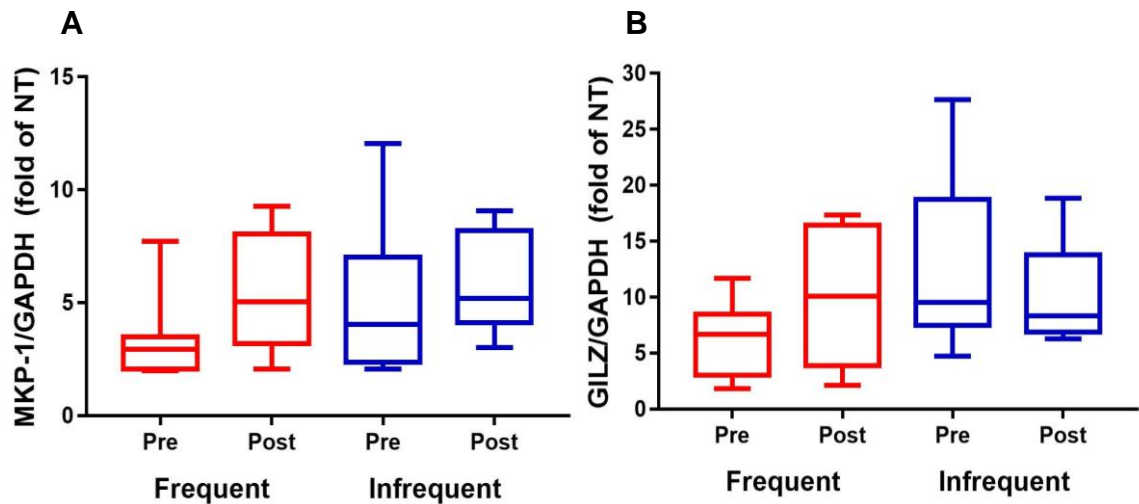


Figure 5.3. Fold changes in MKP-1 (A) and GILZ (B) gene expression from NT in isolated PBMC's following 2 hr of 1µM dexamethasone treatment in frequent and infrequent exacerbators of COPD pre- and post-rehabilitation.

No significant main effects of time were observed with pulmonary rehabilitation and MKP-1 expression following 6 hr of dexamethasone treatment ($p = 0.118$). No significant time \times group interactions ($p = 0.279$) were observed with MKP-1 gene expression following 6 hr treatment with dexamethasone. There was a significant difference between groups whereby frequent exacerbators were observed to have a poorer MKP-1 response to 6 hr dexamethasone treatment compared to infrequent exacerbators across both pre-rehabilitation and post-rehabilitation ($p = 0.003$) (Figure 5.4A).

No main effect of time with pulmonary rehabilitation was observed for GILZ expression following 6 hr treatment with dexamethasone ($p = 0.734$). No significant main time \times group interactions ($p = 0.168$) or differences between groups ($p = 0.151$) were observed for GILZ gene expression following 6 hr treatment with dexamethasone at pre-rehabilitation and post-rehabilitation (Figure 5.4B).

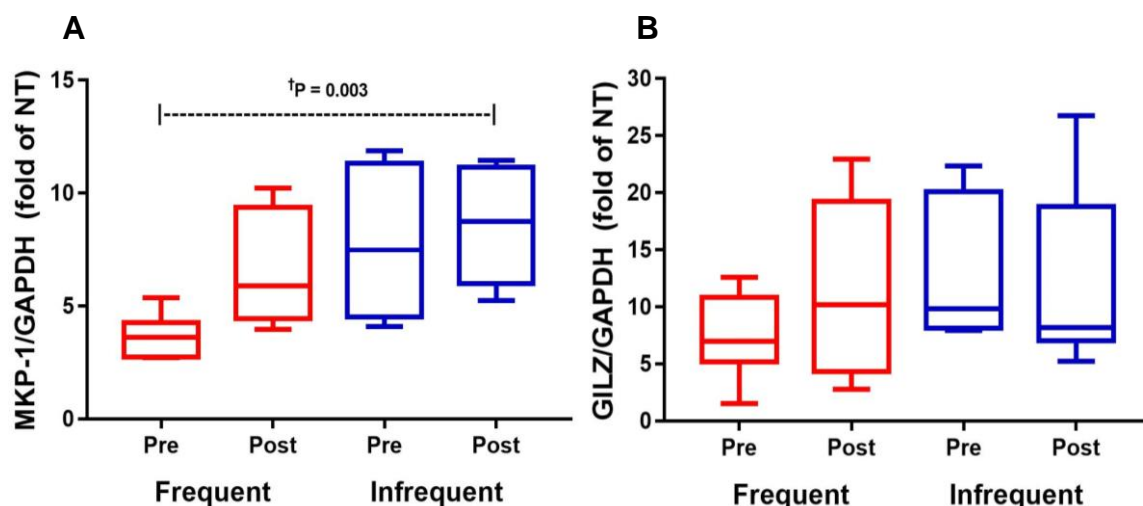


Figure 5.4. Fold changes in MKP-1 (A) and GILZ (B) gene expression from NT in isolated PBMC's following 6 hr of 1µM dexamethasone treatment in frequent and infrequent exacerbators of COPD pre- and post-rehabilitation. [†]Significant difference between groups (p < 0.05).

5.4 Discussion

The main findings from this study suggest that frequent exacerbators may have a poorer response to corticosteroid treatment compared to infrequent exacerbators but that pulmonary rehabilitation may have the potential to modulate MKP-1 expression in response to corticosteroid treatment in both frequent and infrequent exacerbators.

MKP-1 and GILZ are established corticosteroid inducible genes (Kelly et al., 2012; King et al., 2009; Newton & Holden, 2007), and this is the first study to assess how these genes are influenced by a non-pharmacological intervention such as pulmonary rehabilitation. This evidence suggests that pulmonary rehabilitation may play a role in modulating corticosteroid effectiveness in both frequent and infrequent exacerbators of COPD. The tendency towards an upregulation of MKP-1 in response to exercise training draws similarities with previous research in rodents whereby MKP-1 was significantly up-regulated following stimulation at the end of an exercise training programme (Chen, Chen & Jen, 2010). However, the

mechanisms underpinning the upregulation of MKP-1 are currently unclear. One proposed mechanism is that acute exercise temporarily upregulates MKP-1 expression and that regular exercise training may result in a translation of this acute effect into increased basal levels of MKP-1 (Chen, Chen & Jen, 2010). For COPD patients overall, sample size calculations suggest that sample sizes of 55 and 45 would be required to detect statistical significance in MKP-1 expression with 2 hr and 6 hr treatments with fold changes of 1.41 and 1.74 respectively using 80% power and 5% level of significance. For GILZ, sample sizes of 1282 and 661 would be required to detect statistical significance with fold changes of 0.66 and 1.05 for 2 hr and 6 hr treatments respectively. For frequent exacerbators only, sample sizes of 26 and 7 would be required to detect statistical significance in MKP-1 expression with 2 hr and 6 hr treatments with fold changes of 1.81 and 2.91 respectively. Sample sizes of 26 and 32 would be required to detect statistical significance in GILZ expression with 2 hr and 6 hr treatments with fold changes of 3.78 and 4.23 respectively for frequent exacerbators.

The current study provides further evidence of the benefits of pulmonary rehabilitation in the management of COPD by showing potential improvements in pharmacological treatment response. Study 2 highlighted the ability of pulmonary rehabilitation to reduce pro-inflammatory mediators with the current study showing that pulmonary rehabilitation may also play an important role in upregulating anti-inflammatory genes. The implications of this may involve the repurposing of corticosteroids in treatment pathways if levels of reversal of corticosteroid resistance can be achieved, particularly when there has been limited success in establishing anti-inflammatory treatments in COPD (Cazzola et al., 2012). Furthermore, increasing the effectiveness of corticosteroids may also reduce the dosage requirement for treatment responses resultantly reducing the side effects

of corticosteroids (Wood-Baker et al., 2005). Further research needs to confirm the effects of different treatment timepoints and other corticosteroid inducible genes in response to pulmonary rehabilitation. It would also be insightful to assess these responses in the context of early pulmonary rehabilitation where corticosteroids are routinely used during acute exacerbations to see whether pulmonary rehabilitation could have any biological effects that may play a role in improved recovery at a patient level.

For some time, many treatment pathways have recommended the use of ICS in COPD patients who remain breathless or continue to suffer recurrent exacerbations despite LABA therapy (GOLD, 2019). As such ICS use is often observed to be more prevalent in frequent exacerbators (Capozzolo et al., 2017). There are increasing calls, including the updated NICE guidance (NICE, 2018), for prescription of ICS to be limited to COPD patients who present with asthmatic features/corticosteroid responsiveness (e.g. blood eosinophilia) due to the questions over the effectiveness of ICS for reducing exacerbations (Ernst, Saad & Suissa, 2015), and increased risk of pneumonia with long-term ICS use (Suissa et al., 2013). This is the first study to compare corticosteroid effectiveness amongst frequent, infrequent exacerbators and healthy comparators. This study found that corticosteroid treatment induced MKP-1 and GILZ mRNA expression in both COPD patients and healthy controls. This study also suggested that frequent exacerbators had a poorer response to prolonged corticosteroid treatment. Although data presented in this study would suggest that this difference was only apparent with MKP-1 expression and not GILZ. Given the downstream effects of MKP-1 in inhibiting the expression of numerous pro-inflammatory mediators (King et al., 2009), these findings would suggest that corticosteroid treatment has a reduced effectiveness in frequent exacerbators and may in part explain the inability to suppress inflammation

commonly observed in this subset of COPD patients (Hurst et al., 2010). On the other hand, it could also be argued that frequent exacerbators may be less responsive to corticosteroids due to having greater neutrophilic inflammation (Hoenderdos & Condcliffe, 2013). Further research is required to explore the differences in anti-inflammatory gene expression between exacerbation phenotypes of COPD, utilising measures of proteins as well as mRNA.

Questions have also been raised around how long it takes for corticosteroids to take effect and whether COPD patients require a treatment that takes more of an immediate effect (GOLD, 2019). This study is the first study to identify that isolated PBMC's from COPD patients have a differential temporal response to that of healthy individuals. The current study observed that serum starved PBMC's stimulated with corticosteroids from COPD patients experienced an increase in MKP-1 expression between 2 hr and 6 hr treatment points, which was not observed in healthy comparators. This response may indicate that in patients with COPD, corticosteroids take longer to have an effect. The delayed response may go some way to explaining the 'overuse' and developed resistance of ICS in COPD patients with a perceived dependency on effect of the drugs (Koehorst-ter Huurne et al., 2018). This relationship warrants further investigation drawing comparisons between phenotypes.

When interpreting the findings of this study, it is important to consider that these results are based on gene expression within PBMC's only and future research should attempt to assess responses in structural cells where corticosteroids have also been observed to inhibit pro-inflammatory responses (e.g. epithelial and smooth muscle cells) (Ito et al., 2000). However, previous studies have highlighted the usefulness of utilising PBMC's for assessing corticosteroid sensitivity in patients with respiratory disease (Goleva et al., 2012; Hew et al., 2006). It is also important

to consider that no concurrent control group was utilised in this study to confirm the observations seen with pulmonary rehabilitation. These data were provided from mRNA on two corticosteroid inducible genes which provide a limited insight into other corticosteroid inducible pathways in COPD. MKP-1 and GILZ were chosen as they are well-established corticosteroid-inducible genes (Kelly et al., 2012; King et al., 2009; Newton & Holden, 2007), but future research should utilise a wider range of corticosteroid-inducible genes (e.g. regulator of G-protein signalling 2 (RGS2) (Holden et al., 2014)), and assess beta₂-agonist treatments that are also thought to have synergistic effects with ICS on anti-inflammatory pathways (Johnson, 2004).

In conclusion, this study highlighted the potential role that pulmonary rehabilitation can have in increasing the effectiveness of corticosteroids in patients with COPD. This study underlined the poorer expression of MKP-1 in response to corticosteroids in frequent exacerbators of COPD compared to infrequent exacerbators. Further research is required to assess the ability of corticosteroids to activate anti-inflammatory gene pathways in frequent and infrequent exacerbators in response to pulmonary rehabilitation.

Study 4 – Inflammatory responses to acute exercise during pulmonary rehabilitation in patients with COPD

Abstract

Healthy individuals unaccustomed to exercise can experience acute increases in inflammatory mediators in the circulation before an induction of an anti-inflammatory environment with regular bouts of exercise. Short bursts of inflammation in COPD can lead to exacerbations. The aim of this study was to assess the effects of acute exercise on inflammatory parameters in frequent and infrequent exacerbators of COPD at the start (Phase 1) and end (Phase 2) of pulmonary rehabilitation. Blood samples were collected pre- and post-exercise at the 2nd class (Phase 1, n = 40; mild-severe COPD; FEV₁ pred, 51 ± 17%; frequent, n = 26; infrequent, n = 14) and last class (Phase 2, n = 27; frequent, n = 16; infrequent, n = 11) of pulmonary rehabilitation. Circulating fibrinogen & CRP concentrations and total/differential leukocyte counts were measured. Established markers of neutrophil activation (CD11b, CD62L & CD66b) were assessed in blood neutrophils (CD45⁺/CD16b⁺). Respiratory symptoms were assessed pre-rehabilitation and upon commencement of pulmonary rehabilitation via the EXACT[®] questionnaire (Phase 1). Acute exercise (Phase 1) did not result in significant changes in fibrinogen (p = 0.579) or CRP (p = 0.641). Total leukocyte counts were significantly increased following acute exercise in both groups (p = 0.002). Infrequent exacerbators had a significant increase in neutrophil counts following acute exercise (p = 0.001). CD62L expression was significantly reduced following acute exercise in frequent exacerbators (p = 0.009). The proportion of immature neutrophils significantly increased following acute exercise (p = 0.002). There was significant decrease in Cough/Sputum symptoms (p = 0.012) and a tendency

towards a significant decrease in EXACT[®] scores ($p = 0.051$) upon commencement of pulmonary rehabilitation. Pulmonary rehabilitation (Phase 2) did not significantly alter the fibrinogen ($p = 0.089$), CRP ($p = 0.718$), total leukocyte counts ($p = 0.459$), neutrophil counts ($p = 0.769$), CD62L expression ($p = 0.220$), and immature neutrophil ($p = 0.888$) responses to acute exercise. Frequent exacerbators had a significantly decreased CD62L expression in response to acute exercise compared to infrequent exacerbators across both the start, and end of, pulmonary rehabilitation bouts ($p = 0.004$). In conclusion, acute bouts of moderate exercise appear to induce an inflammatory response commonly observed in healthy individuals. Both frequent and infrequent exacerbators appear to adopt largely similar immune responses to acute bouts of exercise, but some inflammatory responses do differ. Preliminary evidence suggests that these immune responses do not appear to correspond to an increase in respiratory symptoms during the early stages of pulmonary rehabilitation. A period of pulmonary rehabilitation does not appear to alter inflammatory responses to acute exercise in frequent or infrequent exacerbators.

6.1 Introduction

Study 1 identified that frequent exacerbators of COPD are less likely to complete pulmonary rehabilitation but achieve clinically significant benefits when doing so. Some qualitative evidence suggests that patients do not attend pulmonary rehabilitation because of a perception of increased respiratory symptoms with exercise (Guo & Bruce, 2014). However, clinical trial and real-life evidence is gathering showing that completion of pulmonary rehabilitation leads to a reduced incidence of exacerbations & hospitalisations (Moore et al., 2016; Steiner et al., 2017a). Study 2 has shed some light on the potential underlying mechanisms, characterised by a reduction in fibrinogen concentration, an established biomarker of exacerbations (Chen, Leung & Sin, 2016). However, the effects of acute bouts of exercise in COPD are unclear whereby short bursts of inflammation resulting from exercise have been proposed to increase the risk of exacerbation (van der Vlist & Janssen, 2010). This may have significant relevance for frequent exacerbators given that they are more susceptible to elevated levels of inflammation at rest (Donaldson et al., 2005a; Perera et al., 2007).

It has previously been suggested that COPD patients avoid exercise due to a dyspnoea-related fear leading to worsening outcomes (Boot, van Exel & van der Gulden, 2009). One potential mechanism for this worsening in outcomes and symptoms may result from the acute inflammatory response to exercise commonly seen in healthy individuals (Walsh et al., 2011), which is becoming increasingly apparent in COPD patients (Jenkins et al., 2015; van der Vlist & Janssen, 2010; van Helvoort et al., 2005). Inflammatory mediators such as fibrinogen and neutrophil count can be seen to be increased post-exercise in healthy and diseased populations (Ahmadizad & El-Sayed, 2005; Jenkins et al., 2015; Li-Saw-Hee et al., 2001; Montgomery et al., 1996; Quindry et al., 2003). In frequent exacerbators,

fibrinogen and neutrophil counts can be seen to be elevated at rest with inflammatory and clinical responses to acute exercise yet to be determined in this phenotype of COPD (Donaldson et al., 2005a; Hurst et al., 2010; Wedzicha, Mackay & Singh, 2013). It has been suggested that responses to acute exercise depend on underlying factors (e.g. training state (Brown et al., 2015)). Some have suggested that acute exercise may be pro-inflammatory in the early stages of an exercise training programme but continuing exercise on a regular basis may favour an anti-inflammatory response (Kasapis & Thompson, 2005).

Previous research has suggested that acute exercise triggers an inflammatory response in healthy individuals as characterised by the mobilisation of neutrophils and increased concentrations of fibrinogen and CRP (Bizheh & Jaafari, 2011; Montgomery et al., 1996; Nieman et al., 2005). In COPD, an acute bout of exercise has been seen to increase neutrophil counts (van Helvoort et al., 2006; Jenkins et al., 2015). However, research assessing the inflammatory responses to acute exercise prior to, during and following pulmonary rehabilitation found that pulmonary rehabilitation did not affect the acute inflammatory responses of CRP (Canavan et al., 2007). This is further supported by Spruit et al. (2007) who found that an acute exercise bout to symptom limitation did not induce significant elevations in CRP or IL-6 in hospitalised and stable COPD patients. Acute bouts of exercise have also been seen to induce changes in the expression of cell surface receptors of neutrophils in healthy populations (Gray et al., 1993; Smith et al., 1996; van Eeden et al., 1999). For example, CD62L expression, which is shed by neutrophils upon activation has been seen to be decreased following exercise indicating an increase in neutrophil activation following exercise (Borregaard & Cowland, 1997; Ley et al., 2007; Nathan, 2006; Wittmann et al., 2004). However, the degree of the inflammatory response to exercise is dependent upon the type,

duration and intensity of exercise (Syu, Chen & Jen, 2012). Such increases in neutrophil activation may have deleterious effects in a population such as COPD that are characterised by neutrophilic inflammation (Hoenderdos & Condliffe, 2013; Quint & Wedzicha, 2007).

It has been suggested that immunological responses to exercise in COPD may hold the key to explaining exacerbation prevention and management (Jenkins, Holden & Jones, 2018). In healthy populations, assessing acute responses to exercise have been suggested to have more clinical significance than training-induced alterations in resting immune/inflammatory parameters (Brandt & Pedersen, 2010). However, the acute effects of exercise on inflammation in COPD patients entering a rehabilitation programme, in particular those patients characterised as having heightened inflammation at rest (i.e. frequent exacerbators) warrant further investigation.

There is a clear lack of evidence assessing the effects of acute exercise, pre- and post-pulmonary rehabilitation, on markers of systemic inflammation in frequent and infrequent exacerbators of COPD. Therefore, this study aimed to assess whether acute exercise in frequent and infrequent exacerbators increases markers of systemic inflammation. This study also aimed to assess whether acute responses to exercise differed following exercise training (e.g. end of a pulmonary rehabilitation programme). It was hypothesised that markers of systemic inflammation would increase following acute exercise during the early stages of pulmonary rehabilitation regardless of exacerbation history. It was also hypothesised that these effects would diminish following an exercise training programme (e.g. pulmonary rehabilitation). In order to achieve these objectives, the study was split into two phases:

- Phase 1 – Immune responses to an initial acute bout of exercise during pulmonary rehabilitation in frequent and infrequent exacerbators of COPD
- Phase 2 – Immune responses to an acute bout of exercise following completion of pulmonary rehabilitation in frequent and infrequent exacerbators of COPD

6.2 Methods

6.2.1 COPD patients

40 COPD patients (age 69 ± 7 years; FEV₁ pred $51 \pm 17\%$; frequent exacerbators = 26; infrequent exacerbators = 14) were recruited.

6.2.2 Procedures

COPD patients performed exercises as part of a pulmonary rehabilitation programme as outlined in section 2.5. Exercise targets were adjusted for each session by the physiotherapist depending on performance, progression and health status of each patient. No dietary interventions or restrictions were put in place for this study.

Blood samples were taken pre- and post-exercise at the beginning (Phase 1, 2nd class) and end (Phase 2, 16th class) of the pulmonary rehabilitation programme, providing patients did not drop-out of the course. The time of blood sample collection was controlled at the 2nd and 16th classes for each patient. The 2nd class of the programme was chosen for assessment of acute responses to exercise as the 1st class of pulmonary rehabilitation is an induction/familiarisation session where the performing of exercises is limited.

The primary outcome (for both phases) of fibrinogen concentration (sodium citrate) and secondary outcome of CRP concentration (K₃EDTA) were quantified in plasma through the use of ELISA's (see section 2.8.2). Secondary outcomes of

total/differential leukocyte counts were quantified via haematological analysis (see section 2.8.1). Further secondary measures were undertaken on a subset of patients whole blood (frequent, n = 21; infrequent, n = 12) to assess markers of neutrophil activation (CD11b, CD62L, CD66b) and neutrophil phenotypes (mature, CD16^{high}/CD62L^{high}; suppressive, CD16^{high}/CD62L^{low}; immature, CD16^{low}/CD62L^{high}; progenitor, CD16^{low}/CD62L^{low}) with the use of flow cytometry (section 2.8.3). For Phase 1, a further subset also consented to completing the EXACT[®] questionnaire daily (section 2.6) in the week leading up to pulmonary rehabilitation (pre-rehabilitation) and the first week of pulmonary rehabilitation (Week 1) (frequent, n = 20; infrequent, n = 11) to quantify changes in respiratory symptoms.

6.2.3 Statistical Analysis

For Phase 1, data were analysed as absolute values (pre- vs post-exercise) whereas Phase 2 data were analysed as mean differences between pre- and post-exercise (beginning vs end of pulmonary rehabilitation). Changes in fibrinogen concentrations, total/differential cell counts, expression of neutrophil activation markers (CD11b, CD62L, CD66b), neutrophil maturity (mature, CD16^{high}/CD62L^{high}; suppressive, CD16^{high}/CD62L^{low}; immature, CD16^{low}/CD62L^{low}; progenitor, CD16^{low}/CD62L^{low}), and EXACT[®] scores (Phase 1 only) including E-RS[™] subset scores (pre-rehabilitation vs. week 1) were analysed using a two-way mixed ANOVA with Bonferroni correction (Phase 1, pre- vs. post-exercise, frequent vs. infrequent; Phase 2, pre- vs. post-rehabilitation, frequent vs. infrequent exacerbators). Any significant main effects observed in the ANOVA were further analysed with post-hoc two-tailed paired t-tests. For Phase 1, changes in CRP concentrations between pre- and post-exercise were analysed using a Wilcoxon

signed rank test with differences between groups at each time point analysed using a Mann-Whitney U test. Statistical significance was accepted at $P < 0.05$.

6.3 Results

6.3.1 Phase 1

6.3.1.1 Patient characteristics

Sodium citrate plasma samples were unable to be obtained from 3 COPD patients for the analysis of fibrinogen (frequent exacerbators, $n = 2$; infrequent exacerbators, $n = 1$) with 1 K₃EDTA plasma sample unable to be obtained for the analysis of CRP (frequent exacerbators, $n = 1$). In the subset of participants who were assessed for neutrophil activation markers, 13 participants were excluded from these analyses due to a lack of expression of CD16b meaning that neutrophils could not be confidently isolated for analysis (frequent exacerbators, $n = 8$; infrequent exacerbators, $n = 5$). Insufficient data point entries (≥ 4 days) were completed for 5 COPD patients with the EXACT[®] questionnaire so these data were not included in the analyses (frequent exacerbators, $n = 4$; infrequent exacerbators, $n = 1$).

The patient characteristics of frequent and infrequent exacerbators are detailed in Table 6.1. All frequent exacerbators were categorised as GOLD grade D (100%) with the majority of infrequent exacerbators classified as GOLD grade B (93%). The majority of frequent and infrequent exacerbators were categorised as either mMRC grade 2, grade 3, or grade 4.

Table 6.1. Patient characteristics

Variable	Frequent	Infrequent
Age (years) ^a	68 ± 6	71 ± 7
% Males ^b	62%	50%
Body Mass (kg) ^a	78 ± 18	76 ± 17
GOLD grade, n (%) ^c		
A	0 (0)	1 (7)
B	0 (0)	13 (93)
C	0 (0)	0 (0)
D	26 (100)	0 (0)
FEV ₁ %predicted ^a	48 ± 16	57 ± 18
Charlson Comorbidity Index ^a	3.7 ± 1.2	4.1 ± 1.1
Current smokers ^b	27%	17%
mMRC, n (%) ^c		
0	0 (0)	1 (7)
1	0 (0)	0 (0)
2	2 (7)	4 (29)
3	9 (35)	3 (21)
4	15 (58)	6 (43)

^aData presented as mean ± SD (t-test). ^bData presented as a % of total population (chi-squared).

^cData presented as total number (% of group). Frequent, n = 26; infrequent, n = 14.

6.3.1.2 Inflammatory responses

There was no significant change in fibrinogen concentrations following an acute bout of exercise at the beginning of pulmonary rehabilitation ($p = 0.579$). No

differences in fibrinogen concentrations were observed between groups ($p = 0.724$) nor were any time \times group interactions seen ($p = 0.204$) (Table 6.2).

Table 6.2. Fibrinogen concentration in response to an acute bout of exercise at the beginning of pulmonary rehabilitation

Variable	Frequent	Infrequent	p-values		
			Time	Group	
			main effect	Time \times group interaction	main effect
Fibrinogen (mg/dL)			0.579	0.724	0.204
Pre-exercise	736 \pm 148	661 \pm 202			
Post-exercise	713 \pm 144	656 \pm 194			

Data presented as mean \pm SD. Fibrinogen: frequent, $n = 24$; infrequent, $n = 13$.

There were no significant differences in CRP concentrations following an acute bout of exercise ($p = 0.264$). CRP concentrations were also not seen to be different between groups at pre-exercise ($p = 0.784$) or post-exercise ($p = 0.965$) (Figure 6.1).

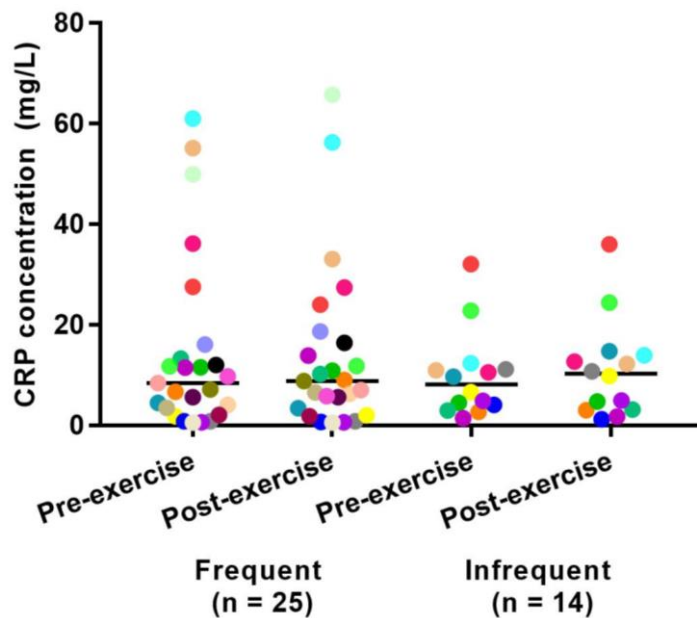


Figure 6.1. CRP concentrations in response to an acute bout of exercise at the beginning of pulmonary rehabilitation in frequent and infrequent exacerbators. Lines represent median values.

There was a significant main effect of time showing an increase in total leukocyte count following an acute bout of exercise ($p = 0.002$). No time \times group interactions were observed with total leukocyte counts ($p = 0.107$). Although not statistically significant, there was a tendency towards a higher total leukocyte count in frequent exacerbators when compared to infrequent exacerbators ($p = 0.069$).

There was a significant main effect of time showing an increase in neutrophil counts post-exercise ($p < 0.001$). A significant time \times group interaction was also seen with neutrophil counts ($p = 0.012$). Post-hoc analyses showed that infrequent exacerbators experienced a significant increase in neutrophil counts ($p = 0.001$) which was not observed in frequent exacerbators ($p = 0.162$). There was a tendency towards a higher neutrophil count in the frequent exacerbators group, but this did not reach statistical significance ($p = 0.088$).

No significant main effects of time were observed for lymphocyte ($p = 0.473$) or eosinophil counts ($p = 0.811$). There were also no significant time \times group interactions for lymphocyte ($p = 0.487$) or eosinophil counts ($p = 0.137$), nor were

any significant differences observed between groups for lymphocyte ($p = 0.611$) or eosinophil counts ($p = 0.286$).

There was a significant main effect of time showing an increase in the neutrophil/lymphocyte ratio following exercise ($p = 0.003$). A significant time \times group interaction was also observed with neutrophil/lymphocyte ratio following acute exercise ($p = 0.007$). Post-hoc analyses showed that infrequent exacerbators experienced a significant increase in neutrophil/lymphocyte ratio following acute exercise ($p = 0.018$) which was not apparent in frequent exacerbators ($p = 0.711$). No significant differences in neutrophil/lymphocyte ratio were seen between frequent and infrequent exacerbators ($p = 0.242$) (Table 6.3).

Table 6.3. Changes in cell counts in response to an acute bout of exercise at the beginning of pulmonary rehabilitation

Variable	Frequent	Infrequent	p-values		
			Time main effect	Time × group interaction	Group main effect
Total Leukocytes (10 ⁹ ·L ⁻¹)			0.002*	0.107	0.069
Pre-exercise	7.9 ± 2.2	6.4 ± 0.9			
Post-exercise	8.1 ± 2.2	7.3 ± 1.3			
Neutrophils (10 ⁹ ·L ⁻¹)			<0.001**	0.012#	0.088
Pre-exercise	4.8 ± 1.7	3.7 ± 0.8			
Post-exercise	5.0 ± 1.8	4.5 ± 1.0			
Eosinophils (10 ⁹ ·L ⁻¹)			0.811	0.137	0.286
Pre-exercise	0.3 ± 0.2	0.2 ± 0.1			
Post-exercise	0.2 ± 0.2	0.2 ± 0.1			
Lymphocytes (10 ⁹ ·L ⁻¹)			0.473	0.487	0.611
Pre-exercise	2.0 ± 0.7	1.9 ± 0.4			
Post-exercise	2.1 ± 0.7	1.9 ± 0.4			
Neutrophil/Lymphocyte ratio			0.003*	0.007#	0.242
Pre-exercise	2.6 ± 1.1	2.0 ± 0.7			
Post-exercise	2.8 ± 1.6	2.4 ± 0.7			

Data presented as mean ± SD. *Significant effect of time (*p < 0.05; ** p < 0.001). #Significant time × group interaction (p < 0.05). Frequent, n = 26; infrequent, n = 14.

6.3.1.3 Neutrophil surface expression

There was no main effect of time on neutrophil CD11b ($p = 0.856$) or CD66b ($p = 0.902$) expression with an acute bout of exercise at the beginning of pulmonary rehabilitation. No time \times group interactions were observed for CD11b ($p = 0.710$) and CD66b ($p = 0.344$). Nor were there any significant differences between frequent and infrequent exacerbators in terms of neutrophil CD11b ($p = 0.465$) and CD66b ($p = 0.699$) expression (Table 6.4).

Table 6.4. Responses of neutrophil activation markers to acute exercise at the beginning of pulmonary rehabilitation

			p-values		
			Time		Group
Neutrophil			main	Time \times group	main
Marker	Frequent	Infrequent	effect	interaction	effect
CD11b (MFI)			0.856	0.710	0.465
Pre-exercise	5401 \pm 707	5707 \pm 955			
Post-exercise	5435 \pm 858	5609 \pm 524			
CD66b (MFI)			0.902	0.344	0.699
Pre-exercise	9506 \pm 2852	9337 \pm 3376			
Post-exercise	9919 \pm 2585	9018 \pm 3634			

Data presented as mean \pm SD. MFI, median fluorescent intensity. Frequent, $n = 13$; infrequent, $n = 7$.

An acute bout of exercise at the beginning of pulmonary rehabilitation did not induce a statistically significant main effect of time on neutrophil CD62L expression ($p =$

0.809). However, significant time \times group interactions were observed with an acute exercise bout ($p = 0.044$). Post-hoc analyses showed that frequent exacerbators experienced a significant decrease in CD62L expression post-exercise ($p = 0.009$), which was not observed in infrequent exacerbators ($p = 0.377$). No significant differences between groups were observed for neutrophil CD62L expression ($p = 0.118$) (Figure 6.2).

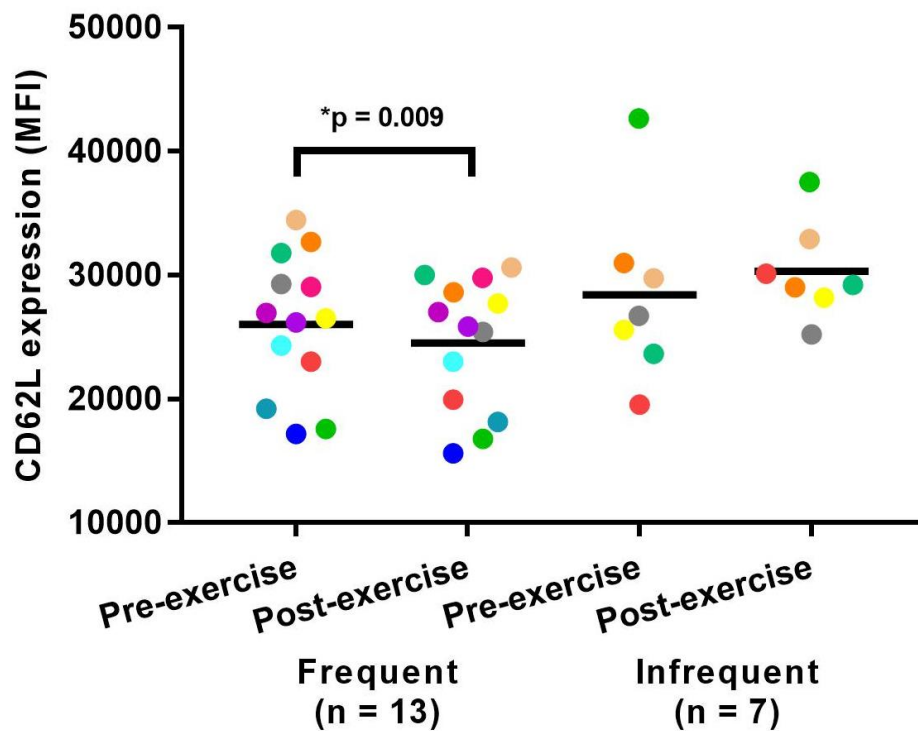


Figure 6.2. Changes in neutrophil CD62L expression following an acute bout of exercise at the beginning of pulmonary rehabilitation in frequent and infrequent exacerbators. Lines represent mean values. *Significant difference between pre- and post-exercise ($p < 0.05$).

There were no significant main effects of time on mature ($CD16b^{high}/CD62L^{high}$) ($p = 0.799$), suppressive ($CD16b^{high}/CD62L^{low}$) ($p = 0.272$), or progenitor ($CD16b^{low}/CD62L^{low}$) ($p = 0.544$) neutrophil subsets following an acute bout of exercise. There were also no significant time \times group interactions (mature, $p = 0.856$; suppressive, $p = 0.533$; progenitor, $p = 0.765$) or differences between groups (mature, $p = 0.928$; suppressive, $p = 0.823$; progenitor, $p = 0.898$) (Table 6.5).

Table 6.5. Neutrophil phenotypes in response to acute exercise at the beginning of pulmonary rehabilitation

Neutrophil Marker	Frequent	Infrequent	p-values		
			Time	Time × group	Group
			main effect	interaction	main effect
CD16b ^{high} /CD62L ^{high} (%)			0.799	0.856	0.928
Pre-exercise	92.7 ± 3.6	92.6 ± 1.9			
Post-exercise	92.6 ± 2.7	92.4 ± 0.8			
CD16b ^{high} /CD62L ^{low} (%)			0.272	0.533	0.823
Pre-exercise	5.7 ± 3.7	5.3 ± 2.4			
Post-exercise	5.2 ± 2.8	4.6 ± 2.0			
CD16b ^{low} /CD62L ^{low} (%)			0.554	0.765	0.898
Pre-exercise	0.2 ± 0.1	0.1 ± 0.0			
Post-exercise	0.2 ± 0.1	0.2 ± 0.3			

Data presented as mean ± SD. Frequent, n = 13; infrequent, n = 7.

A significant main effect of time showing an increase in the percentage of immature (CD16b^{low}/CD62L^{high}) neutrophils was observed post-exercise ($p = 0.002$). No time and group interactions ($p = 0.691$) or differences between groups ($p = 0.176$) were observed with an acute exercise bout for the immature neutrophil subset (Figure 6.3).

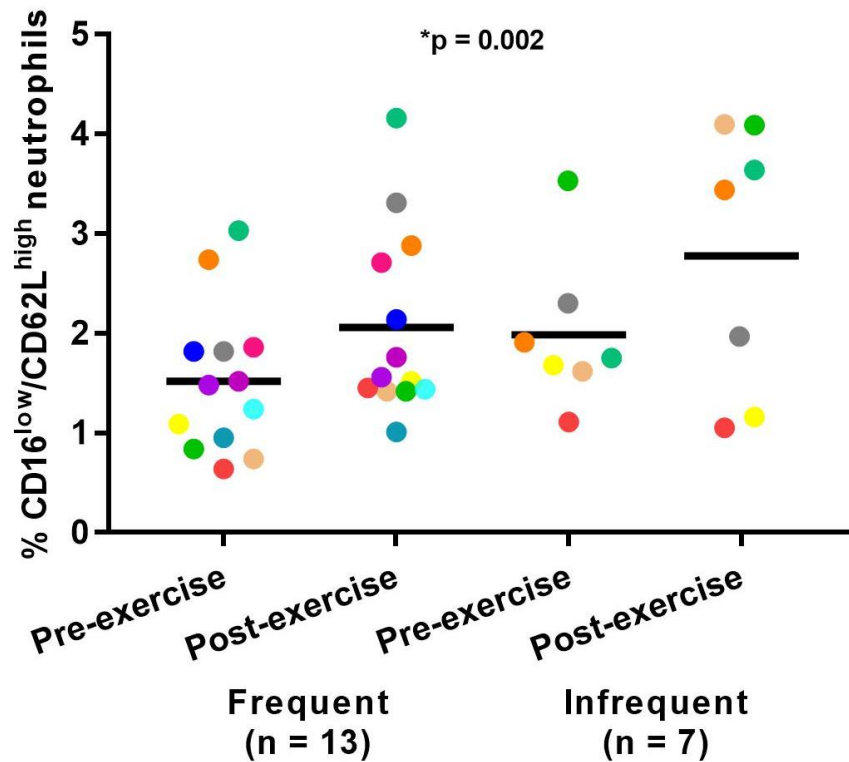


Figure 6.3. Changes in percentage of immature neutrophils following an acute bout of exercise at the beginning of pulmonary rehabilitation in frequent and infrequent exacerbators. Lines represent mean values. *Significant main effect of time ($p < 0.05$).

6.3.1.4 EXACT[®] and E-RS[™]

A tendency towards a decrease in total EXACT[®] score was observed in both groups following the commencement of pulmonary rehabilitation ($p = 0.051$). There was no significant time \times group interactions observed ($p = 0.670$), nor any significant differences between groups ($p = 0.217$). An example of daily reported EXACT[®] scores between pre-rehab and week 1 for one patient is provided (Figure 6.4).

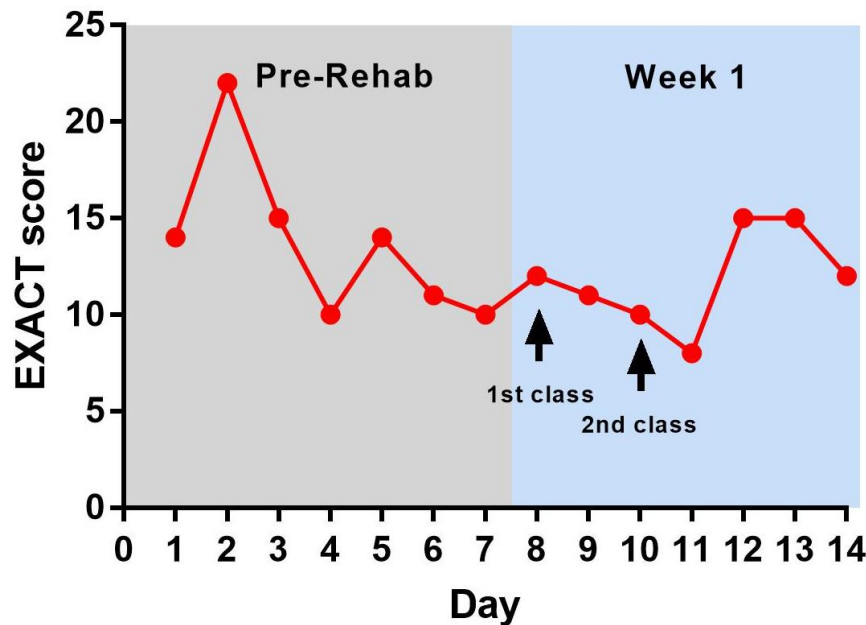


Figure 6.4. An example of daily EXACT[®] scores for one patient in the week prior to (Pre-Rehab), and week of (Week 1), commencing pulmonary rehabilitation.

There was a tendency towards a decrease in E-RS[™] score in both groups upon commencement of pulmonary rehabilitation, but this was not found to be statistically significant ($p = 0.083$). No time \times group interactions ($p = 0.667$) or differences between groups ($p = 0.296$) were observed with E-RS[™] score.

There were no significant effects of time on breathlessness ($p = 0.515$) or chest symptoms ($p = 0.560$) between pre-rehabilitation and week 1. No significant time \times group interactions (breathlessness, $p = 0.686$; chest, $p = 0.649$) or differences between groups (breathlessness, $p = 0.391$; chest, $p = 0.488$) were observed.

There was a significant main effect of time showing a reduction in cough/sputum symptoms upon commencing pulmonary rehabilitation ($p = 0.012$). No significant time \times group interactions ($p = 0.567$) or differences between groups ($p = 0.090$) were observed (Table 6.6).

Table 6.6. Patient reported EXACT® scores between pre-rehabilitation and week 1

EXACT® domain	Frequent	Infrequent	p-values		
			Time main effect	Time x group interaction	Group main effect
EXACT®-Total score			0.051	0.670	0.217
Pre-Rehab	19.3 ± 10.6	14.6 ± 5.7			
Week 1	18.1 ± 10.8	13.7 ± 6.7			
E-RS™-Total score			0.083	0.667	0.296
Pre-Rehab	15.5 ± 8.2	12.4 ± 4.9			
Week 1	14.7 ± 8.4	11.8 ± 5.5			
Breathlessness score			0.515	0.686	0.391
Pre-Rehab	7.8 ± 4.8	6.5 ± 3.2			
Week 1	7.7 ± 4.9	6.4 ± 3.1			
Cough/Sputum score			0.012*	0.567	0.090
Pre-Rehab	3.0 ± 0.8	2.6 ± 0.4			
Week 1	2.8 ± 1.0	2.2 ± 0.6			
Chest score			0.560	0.649	0.488
Pre-Rehab	3.3 ± 2.2	2.6 ± 1.7			
Week 1	3.1 ± 2.2	2.6 ± 2.0			

Data presented as mean ± SD. *Significant main effect of time (p < 0.05). Frequent, n = 16; infrequent, n = 10.

6.3.2 Phase 2

6.3.2.1 Patient characteristics

There were 13 COPD patients lost due to follow-up (frequent exacerbators, n = 10; infrequent exacerbators, n = 3) at the end of pulmonary rehabilitation. Sodium

citrate plasma samples were unable to be obtained from 2 COPD patients for the measurement of fibrinogen (frequent exacerbators, n = 1; infrequent exacerbators, n = 1). Four COPD patients were lost in the follow-up for the measurement of neutrophil activation markers (frequent exacerbators, n = 2; infrequent exacerbators, n = 2).

The patient characteristics of frequent and infrequent exacerbators are detailed in Table 6.7. All frequent exacerbators were categorised as GOLD grade D (100%) with the majority of infrequent exacerbators classified as GOLD grade B (91%). The majority of frequent and infrequent exacerbators were categorised as either mMRC grade 2, grade 3, or grade 4.

Table 6.7. Patient characteristics

Variable	Frequent	Infrequent
Age (years) ^a	69 ± 7	69 ± 6
% Males ^b	63%	45%
Body Mass (kg) ^a	76 ± 15	72 ± 16
GOLD grade, n (%) ^c		
A	0 (0)	1 (9)
B	0 (0)	10 (91)
C	0 (0)	0 (0)
D	16 (100)	0 (0)
FEV ₁ %predicted ^a	48 ± 18	53 ± 15
Charlson Comorbidity Index ^a	4.0 ± 1.3	4.0 ± 1.2
Current smokers ^b	19%	18%
MRC, n (%) ^c		
0	0 (0)	1 (9)
1	0 (0)	0 (0)
2	1 (6)	3 (27)
3	7 (44)	2 (18)
4	8 (50)	5 (46)

^aData presented as mean ± SD (t-test). ^bData presented as a % of total population (chi-squared).

^cData presented as total number (% of group). Frequent, n = 16; infrequent, n = 11.

6.3.2.2 Inflammatory responses

There was a tendency towards an effect of condition ($p = 0.089$) on fibrinogen concentration but this was not found to be statistically significant. There were no

significant condition \times group interactions ($p = 0.705$) or differences between groups ($p = 0.098$) when comparing fibrinogen responses to acute exercise between bouts taking place at the start and end of pulmonary rehabilitation.

No significant effects of condition ($p = 0.718$) or condition \times group interaction ($p = 0.616$) were observed when comparing CRP responses to acute exercise between bouts taking place at the start and end of pulmonary rehabilitation. There was a tendency towards a difference between groups but this was not statistically significant ($p = 0.064$) (Table 6.8).

Table 6.8. Comparisons of systemic inflammatory responses to acute bouts of exercise at the beginning and end of pulmonary rehabilitation

Variable	Frequent	Infrequent	p-values		
			Condition main effect	Condition \times group interaction	Group main effect
Fibrinogen (mg/dL)			0.089	0.705	0.098
2 nd class (pre vs post)	-57 \pm 146	-14 \pm 189			
16 th class (pre vs post)	12 \pm 112	92 \pm 160			
CRP (mg/L)			0.718	0.616	0.064
2 nd class (pre vs post)	0.5 \pm 5.3	1.5 \pm 2.2			
16 th class (pre vs post)	-0.8 \pm 5.7	3.1 \pm 7.2			

Data expressed as mean differences \pm SD between pre- and post-exercise at the 2nd and 16th classes. Fibrinogen: frequent, $n = 15$; infrequent, $n = 10$. CRP: frequent, $n = 16$; infrequent, $n = 11$.

No significant effects of condition were observed for total leukocyte count ($p = 0.459$), neutrophil count ($p = 0.769$), lymphocyte count ($p = 0.280$), and neutrophil/lymphocyte ratio ($p = 0.510$) when comparing responses to acute exercise between bouts taking place at the start and end of pulmonary rehabilitation. There were also no significant condition \times group interactions (total leukocyte, $p = 0.156$; neutrophil, $p = 0.197$; lymphocyte, $p = 0.736$; neutrophil/lymphocyte, $p = 0.278$) or differences between groups (total leukocyte, $p = 0.552$; neutrophil, $p = 0.150$; lymphocyte, $p = 0.105$; neutrophil/lymphocyte, $p = 0.283$).

No significant effect of condition was observed for eosinophil count ($p = 0.532$) when comparing responses to acute exercise bouts at the start and end of pulmonary rehabilitation. However, a significant condition \times group interaction ($p = 0.012$) was observed. Post-hoc analyses showed that frequent exacerbators had a significantly different response to acute exercise between bouts taking place at the start and end of pulmonary rehabilitation as characterised by an increase in eosinophil count in response to acute exercise at the end of pulmonary rehabilitation, as opposed to a decrease observed at the start ($p = 0.025$). No significant differences between conditions for eosinophil count were observed for infrequent exacerbators ($p = 0.180$). No significant group differences were observed for eosinophil count ($p = 0.733$) (Table 6.9).

Table 6.9. Comparisons of mean differences in blood count responses to acute bouts of exercise at the beginning and end of pulmonary rehabilitation

Variable	Frequent	Infrequent	p-values		
			Condition main effect	Condition × group interaction	Group main effect
Total Leukocytes (10 ⁹ ·L ⁻¹)			0.459	0.156	0.552
2 nd class (pre vs post)	0.10 ± 1.32	0.75 ± 0.94			
16 th class (pre vs post)	0.81 ± 1.08	0.52 ± 0.96			
Neutrophils (10 ⁹ ·L ⁻¹)			0.769	0.197	0.150
2 nd class (pre vs post)	0.11 ± 0.91	0.80 ± 0.82			
16 th class (pre vs post)	0.51 ± 0.80	0.54 ± 0.80			
Eosinophils (10 ⁹ ·L ⁻¹)			0.532	0.012 [#]	0.733
2 nd class (pre vs post)	-0.02 ± 0.05	0.01 ± 0.03			
16 th class (pre vs post)	0.01 ± 0.03	-0.01 ± 0.02			
Lymphocytes (10 ⁹ ·L ⁻¹)			0.280	0.736	0.105
2 nd class (pre vs post)	0.04 ± 0.28	-0.09 ± 0.37			
16 th class (pre vs post)	0.15 ± 0.28	-0.03 ± 0.31			
Neutrophil/Lymphocyte ratio			0.510	0.278	0.283
2 nd class (pre vs post)	0.14 ± 0.63	0.45 ± 0.58			
16 th class (pre vs post)	0.18 ± 0.53	0.26 ± 0.60			

Data expressed as mean differences ± SD between pre- and post-exercise at the 2nd and 16th classes. [#]Significant condition × group interaction (p < 0.05). Frequent, n = 16; infrequent, n = 11.

6.3.2.3 Neutrophil surface expression

No significant effects of condition were observed for CD11b ($p = 0.390$), CD62L ($p = 0.220$) and CD66b expression ($p = 0.810$) when comparing responses between acute bouts of exercise at the start and end of pulmonary rehabilitation. No significant condition \times group interactions (CD11b, $p = 0.568$; CD62L, $p = 0.251$; CD66b, $p = 0.455$) were observed. There was a significant difference between groups for CD62L expression whereby frequent exacerbators had significantly decreased CD62L expression in response to acute exercise ($p = 0.004$). No significant differences between groups were observed for CD11b ($p = 0.831$) and CD66b ($p = 0.636$) expression (Table 6.10).

Table 6.10. Comparison of mean differences for neutrophil activation markers in response to acute exercise at the beginning and end of pulmonary rehabilitation

			p-values		
			Condition	Condition	Group
Neutrophil			main	× group	main
Marker	Frequent	Infrequent	effect	interaction	effect
CD11b (MFI)			0.390	0.568	0.831
2 nd class	17 ± 780	215 ± 460			
(pre vs post)					
16 th class	-51 ± 696	-115 ± 744			
(pre vs post)					
CD62L (MFI)			0.220	0.251	0.004 [†]
2 nd class	-1734 ± 1771	4083 ± 4431			
(pre vs post)					
16 th class	-1841 ± 3873	1002 ± 3082			
(pre vs post)					
CD66b (MFI)			0.810	0.455	0.636
2 nd class	364 ± 2029	-304 ± 475			
(pre vs post)					
16 th class	149 ± 1311	111 ± 1432			
(pre vs post)					

Data expressed as mean differences ± SD between pre- and post-exercise at the 2nd and 16th classes. [†]Significant main difference between groups (p < 0.05). MFI, median fluorescent intensity. Frequent, n = 11; infrequent, n = 5.

No significant effects of condition were observed for mature (CD16b^{high}/CD62L^{high}) (p = 0.162), immature (CD16b^{low}/CD62L^{high}) (p = 0.888), suppressive (CD16b^{high}/CD62L^{low}) (p = 0.114), or progenitor (CD16b^{low}/CD62L^{low}) (p = 0.964)

neutrophil subsets when comparing between acute bouts of exercise at the start and end of pulmonary rehabilitation. No significant condition \times group interactions were observed for mature ($p = 0.633$), immature ($p = 0.639$), suppressive ($p = 0.722$), or progenitor ($p = 0.876$) neutrophil subsets. No significant differences between groups were observed for mature ($p = 0.669$), immature ($p = 0.586$), suppressive ($p = 0.892$), or progenitor ($p = 0.939$) neutrophil subsets when comparing responses to acute exercise at the start and end of pulmonary rehabilitation (Table 6.11).

Table 6.11. Comparison of neutrophil phenotypes in response to acute bouts of exercise at the beginning and end of pulmonary rehabilitation

			p-values		
Neutrophil Marker	Frequent	Infrequent	Condition	Condition	Group
			main	× group	main
			effect	interaction	effect
CD16b ^{high} /CD62L ^{high} (%)			0.162	0.633	0.669
2 nd class (pre vs post)	-0.14 ± 2.12	-0.16 ± 2.01			
16 th class (pre vs post)	-0.75 ± 1.58	-1.37 ± 0.94			
CD16b ^{low} /CD62L ^{high} (%)			0.888	0.639	0.586
2 nd class (pre vs post)	0.54 ± 0.46	0.69 ± 1.39			
16 th class (pre vs post)	0.45 ± 0.46	0.74 ± 1.17			
CD16b ^{high} /CD62L ^{low} (%)			0.114	0.722	0.892
2 nd class (pre vs post)	-0.42 ± 2.06	-0.52 ± 1.76			
16 th class (pre vs post)	0.31 ± 1.52	0.62 ± 1.21			
CD16b ^{low} /CD62L ^{low} (%)			0.964	0.876	0.939
2 nd class (pre vs post)	0.00 ± 0.15	0.00 ± 0.07			
16 th class (pre vs post)	-0.01 ± 0.33	0.01 ± 0.03			

Data expressed as mean differences ± SD between pre- and post-exercise at the 2nd and 16th classes. Frequent, n = 11; infrequent, n = 5.

6.4 Discussion

The main findings from this study suggest that acute exercise in frequent and infrequent exacerbators at the start of pulmonary rehabilitation does not result in significant increases in the concentrations of fibrinogen and CRP. However, increases in total leukocyte counts and circulating immature neutrophils are observed following this initial acute bout of exercise. These acute inflammatory

responses to exercise did not correspond to increases in respiratory symptoms in either group. Following a period of pulmonary rehabilitation, no significant changes in fibrinogen, CRP, total leukocyte, and neutrophil count responses to acute exercise were observed. Comparisons between acute bouts at the start and end of pulmonary rehabilitation also showed that frequent exacerbators had significantly decreased CD62L responses compared to infrequent exacerbators.

This is the first study in the COPD population to assess fibrinogen concentrations in response to acute exercise bouts at the beginning and end of pulmonary rehabilitation. The present findings suggest that acute bouts of exercise in the context of a traditional pulmonary rehabilitation programme are not sufficient to significantly alter the concentrations of fibrinogen. Of the limited evidence available assessing fibrinogen responses to acute exercise, the present findings disagree with previous research in other clinical populations (e.g. atrial fibrillation) (Li-Saw-Hee et al., 2001), whereby fibrinogen levels were observed to increase following an acute bout of exercise. It is important to note that others have utilised strenuous exercise to assess fibrinogen responses (Li-Saw-Hee et al., 2001). It has previously been suggested that acute exercise, especially if prolonged or highly intense, can induce inflammation (Woods et al., 2012). The current study did not control for exercise intensity but conceivably, at least at the start of pulmonary rehabilitation, absolute exercise intensity was low. Therefore, it may be deemed that exercise needs to be strenuous, or prolonged in duration, to induce increases in fibrinogen concentration. Nevertheless, the findings indicate that acute exercise as part of pulmonary rehabilitation, even at end of the programme, does not exacerbate key markers of systemic inflammation in either frequent or infrequent exacerbators of COPD. Further research is warranted to confirm whether fibrinogen responses to

acute exercise in COPD, may differ if exercise or pulmonary rehabilitation programmes prescribe higher exercise intensities.

This study also found that CRP was not significantly elevated following acute bouts of exercise at the start and end of pulmonary rehabilitation. This agrees with previous research in the context of pulmonary rehabilitation whereby CRP was not seen to be elevated following acute bouts of exercise at the beginning and end of pulmonary rehabilitation (Canavan et al., 2007). The conclusions from the Canavan et al. (2007) study suggested that pulmonary rehabilitation was unlikely to enhance systemic inflammation. The findings also agree with previous research utilising an acute exercise bout to symptom limitation outside of the context of pulmonary rehabilitation in hospitalised and stable COPD patients whereby no significant increases in CRP were observed post-exercise (Spruit et al., 2007). In the healthy population, a systematic review has demonstrated inconsistent effects of bouts of acute exercise on CRP concentrations (Brown et al., 2015). In COPD, the available evidence to date, suggests a lack of effect of acute exercise on CRP concentrations. However, it is important to note that CRP was highly variable between patients in the current study demonstrating the differing degrees of systemic inflammation present in patients with COPD (Study 2; Aksu et al., 2013; Silva, Gazzana & Knorst, 2015), and this may in part explain any lack of effect observed with acute exercise. Research should assess the effects of different modalities of acute exercise on CRP concentrations before any firm conclusions can be drawn.

Although inflammation as characterised by fibrinogen and CRP concentrations was not increased with acute exercise, current study findings demonstrated changes in immune cell trafficking, regardless of whether the bout was at the beginning or end of pulmonary rehabilitation, as defined by increases in total leukocyte counts in both

groups and neutrophil counts in infrequent exacerbators only. This agrees with well-established literature assessing acute inflammatory responses to exercise in the healthy population (Brown et al., 2015; Nieman et al., 1991; Quindry et al., 2003), and the limited existing evidence in COPD populations (Jenkins et al., 2015; Menon et al., 2012; van Helvoort et al., 2005). While it is not surprising to see these effects, it could be hypothesised that the acute inflammatory response to exercise could diminish by the end of pulmonary rehabilitation as a result of exercise training (Beavers, Brinkley & Nicklas, 2010). This was not observed in the current study which may suggest that despite the absolute exercise intensity being lower at the start of pulmonary rehabilitation, the programme in the current study was structured in such a way (progressive programme (Singh & Steiner, 2013)), that the relative exercise intensity remains the same. Resultantly, the stress applied on the immune system is similar on an individual basis with each exercise class of pulmonary rehabilitation. However, it would be insightful to assess inflammatory responses to two controlled bouts of exercise, in terms of intensity and duration, before and after an exercise training programme as recently undertaken (de Alencar Silva et al., 2018). The findings of de Alencar Silva et al. (2018) refute the findings proposed here as it was observed that 12 weeks of exercise training reduced the acute inflammatory responses to exercise. It is important to note that the aforementioned study utilised a longer exercise programme, in terms of duration, and controlled the acute exercise bouts when comparing findings to that of the current study.

Despite no significant changes in neutrophil counts with acute exercise in frequent exacerbators, there was a significant reduction in CD62L expression post-exercise. These responses were not observed in infrequent exacerbators, who experienced an increase in neutrophil count with acute exercise. The findings observed with frequent exacerbators in the current study agree with that of previous literature

suggesting a reduction in CD62L expression is commonly seen post-exercise (Kurokawa et al., 1995; van Eeden et al., 1999). This observed response in frequent exacerbators is typically indicative of an increased activation of neutrophils (Figure 1.1; Ley et al., 2007). An increased activation of neutrophils may in part be explained by the acute inflammatory response to exercise whereby neutrophils activate to release free radicals which aid in the clearance of damaged host tissue (Peake & Suzuki, 2004; Peake, 2002). CD62L is a marker for adhesion of neutrophils to endothelial cells (Monteseirin et al., 2005) which may suggest that frequent exacerbators are experiencing more firm adhesion following exercise and possible migration through tissue to sites of inflammation (Figure 1.1; Ley et al., 2007). The interpretation of this is open to discussion, as the inflammatory response could be deemed as 'good' due to the need to activate the immune system to resolve host tissue damage commonly experienced with exercise (Peake & Suzuki, 2004; Peake, 2002). However, it could also be deemed as 'bad' due to the inflammatory nature of COPD and the frequent exacerbator phenotype, whereby acute increases in inflammation may contribute towards more chronic inflammatory processes (Oudijk, Lammers & Koenderman, 2003). However, when taking into account the EXACT® & E-RS™ data, this would suggest that in the initial phases these changes do not appear to increase respiratory symptoms. With regards to CD11b and CD66b, the current research disagrees with previous research in a healthy population which demonstrated an increase in CD11b expression post-exercise (Pizza et al., 1996; Smith et al., 1996; van Eeden et al., 1999), with similar observations seen in a clinical population of claudicant patients (Nawaz et al., 2001; Turton et al., 1998). Previous research has also demonstrated that an acute bout of exercise induces increases in CD66b expression (Nawaz et al., 2001), conflicting with the findings observed in the current study. However, in the Nawaz et al. (2001)

study, such observations were only seen in the lower limb exercise group and not in the upper limb exercise group demonstrating an important potential effect of modality of exercise. The lack of effects seen with CD11b in the upper limb exercise group (Nawaz et al., 2001), agrees with the findings of the current study. There is a need to further understand neutrophil activation responses to acute exercise in frequent and infrequent exacerbators of COPD and determine clinical effects of such changes.

This study was also the first study to utilise the newly proposed phenotyping approach of neutrophils in an exercise setting with a COPD population (Cortjens et al., 2017). An acute bout of exercise was seen to increase the percentage of immature neutrophils in both groups following exercise at the beginning of pulmonary rehabilitation with similar responses observed following completion of the programme. No concomitant changes were observed amongst the remaining neutrophil phenotypes as a result of this change. This approach has not yet been adopted in the field of exercise immunology so comparative literature is not yet available. However, these results support the notion long proposed in healthy populations whereby the physical stress of exercise induces an inflammatory response and a temporary neutrophilia, with increased release of immature neutrophils from the bone marrow (Suzuki et al., 1999; Yamada et al., 2002), which is more exaggerated in untrained individuals (Risoy et al., 2003). This increased release of immature neutrophils from the bone marrow has been proposed to be down to the need to compensate for the exhaustion of mature neutrophils from the marginated pools during, and following exercise which is typically observed and robust irrespective of the population of interest (Suzuki et al., 1999; Yamada et al., 2002). This study has demonstrated this is apparent in both frequent and infrequent exacerbators of COPD with infrequent exacerbators experiencing this in the

presence of an increase in neutrophil count post-exercise. It would be insightful to utilise this phenotypic approach in future to further explore the effects of acute exercise on neutrophil phenotypes.

Although measuring the responses of inflammatory markers to acute exercise provides mechanistic explanations, it could be argued that this provides limited information on the effects of exercise on patient reported symptoms. This was the first study to utilise the EXACT® questionnaire in the context of pulmonary rehabilitation for monitoring of daily respiratory symptoms. This study found that commencing a pulmonary rehabilitation programme did not increase EXACT® scores and respiratory symptoms despite acute inflammatory responses to exercise. In fact, this study demonstrated a tendency towards a decrease in respiratory symptoms upon commencing pulmonary rehabilitation. This is positive given that acute inflammatory responses to exercise have been linked with increased susceptibility to symptoms of infection (Walsh et al., 2011). However, increased susceptibility to respiratory symptoms may also arise from factors such as the environment (e.g. dry/cold air) and/or exercise-induced asthma rather than an actual infectious event (Walsh et al., 2011). Interestingly, the most pronounced effects of exercise on respiratory symptoms were seen in the Cough/Sputum domain suggesting that commencing pulmonary rehabilitation may play a role in airway clearance, agreeing with previous recommendations for utilising exercise as a form of airway clearance (Bott et al., 2009). Although these findings were only observed in a subset of patients, it may suggest that exercise, in the context of a pulmonary rehabilitation programme, may lead to early decreases in respiratory symptoms. Further exploration of respiratory symptom monitoring in the context of pulmonary rehabilitation is required assessing potential mechanisms for decreases in symptoms.

This study implies that acute exercise does not appear to worsen inflammation in frequent and infrequent exacerbators as characterised by a lack of increase in established biomarkers of exacerbations (fibrinogen & CRP). Acute exercise does appear to induce a classical inflammatory response to exercise, as observed previously in healthy (Brown et al., 2015; Nieman et al., 1991; Quindry et al., 2003), and COPD populations (Jenkins et al., 2015; Menon et al., 2012; van Helvoort et al., 2005), as characterised by increases in total leukocyte counts. This inflammatory response did not correspond to an increase in respiratory symptoms, in fact, there was tendency towards an immediate reduction in respiratory symptoms upon commencing the pulmonary rehabilitation programme. Importantly, this study suggested that a period of pulmonary rehabilitation was insufficient to arrest observed inflammatory responses to acute bouts of exercise. These findings warrant further follow-up assessing acute inflammatory responses to exercise and how they correspond to reported respiratory symptoms in frequent and infrequent exacerbators of COPD.

When interpreting the findings of the current study, it is important to consider several limiting factors. Some potentially important cofounders were not accounted for such as exercise duration and intensity of both groups, and current physical activity status of the individuals taking part, whereby physically active lifestyles have been seen to alleviate some of the inflammatory responses seen with an acute bout of exercise (Gokhale, Chandrashekara & Vasanthakumar, 2007; Woods et al., 2012). Research should take these into account when assessing responses amongst differing phenotypes of COPD before conclusions can be made. Another important factor to consider is that the acute exercise bouts were not controlled at the beginning and end of pulmonary rehabilitation. The duration and absolute intensity were not the same between the two timepoints, but the modality of

exercise and relative intensity were kept consistent. Finally, the sample size used to make inferences about the neutrophil activation markers was impacted by an unforeseen deficiency of CD16b expression in subsets of patients meaning the results were underpowered. This reduced the confidence to be able to separate neutrophils from eosinophils, and research has previously suggested two possible causes; a rare hereditary deficiency of CD16 (Wagner & Hansch, 2004), or a shedding of CD16b via the action of ADAM17 (Wang et al., 2013). These proposals could not be assessed in the current study and future studies should be wary of the use of CD16b as a marker for identifying neutrophils.

In conclusion, acute bouts of exercise at the beginning and end of pulmonary rehabilitation do not appear to alter fibrinogen or CRP concentrations but do induce increases in total leukocyte counts. Increases in total leukocytes counts with exercise is accompanied by an increase in the percentage of immature neutrophils in circulation. Frequent exacerbators appear to respond differently, in terms of reductions in neutrophil CD62L expression, following acute exercise. Future research should attempt to further determine the clinical implications of such changes in inflammatory parameters with acute exercise in frequent and infrequent exacerbators of COPD.

Study 5 – A pilot study assessing physical activity levels between frequent and infrequent exacerbators of COPD following pulmonary rehabilitation

Abstract

Pulmonary rehabilitation is considered to be ineffective at inducing long-term increases in physical activity levels of COPD patients. Exacerbations of COPD have been associated with reduced physical activity levels. It is unclear whether frequent exacerbators of COPD are less physically active than infrequent exacerbators. The primary aim of this pilot study was to explore physical activity in frequent exacerbators of COPD. The secondary aim of this pilot study was to explore inflammatory profiles of frequent and infrequent exacerbators following pulmonary rehabilitation. COPD patients (frequent, $n = 9$; infrequent, $n = 9$; moderate-very severe COPD; FEV₁ pred, 52 ± 15) who had previously enrolled onto pulmonary rehabilitation (>3 months) were recruited for 7 days of physical activity monitoring (Actigraph wGT3X-BT). Daily step counts were recorded and physical activity levels were categorised as sedentary (0 to 1.5 metabolic equivalent of task (METs)), light (1.5 to 3 METs), and moderate-to-vigorous (>3 METs). % of time spent in standing, seated, and lying positions were also recorded. Blood samples were collected and analysed for circulating fibrinogen & CRP concentrations, total/differential cell counts, and anti-inflammatory gene expression (MKP-1 & GILZ). Effects sizes were calculated for outcomes and classified as small ($d = 0.2$), medium ($d = 0.5$), or large ($d = 0.8$). Frequent exacerbators recorded fewer daily steps ($d = 0.3$) and spent less time in light ($d = 0.8$) and moderate-to-vigorous activities ($d = 0.3$). Trivial differences between groups were observed for time spent in sedentary activities ($d = 0.1$). Frequent exacerbators spent less time in the standing position ($d = 0.4$) and more time in seated positions ($d = 0.6$). Fibrinogen ($d = 0.3$) and CRP ($d = 0.2$) were

observed to be elevated in frequent exacerbators. Total leukocyte ($d = 0.6$), neutrophil ($d = 0.5$), and lymphocyte counts ($d = 0.7$) were observed to be lower in frequent exacerbators. Lower GILZ expression in response to dexamethasone treatment was observed in frequent exacerbators (2 hr, $d = 0.8$; 6 hr, $d = 0.5$). This pilot study suggests that frequent exacerbators are less physically active and spend more time in sedentary positions following pulmonary rehabilitation. Frequent exacerbators appeared to have heightened levels of known biomarkers for exacerbations and poorer response to corticosteroid treatment. Larger-scale trials are required assessing physical activity in the frequent exacerbator phenotype.

7.1 Introduction

In healthy individuals, physical activity levels have been shown to contribute to the primary and secondary prevention of several chronic diseases such as COPD (Warburton, Nicol & Bredin, 2006). In comparison to healthy counterparts, COPD patients present with decreased daily physical activity levels and increased time spent in a sedentary state (Pitta et al., 2005). This is believed to be due to the nature of disease whereby patients limit their physical activity to avoid symptoms such as dyspnoea (Giacomini et al., 2012). Pulmonary rehabilitation is designed to provide COPD patients with the tools to be able to translate increases in exercise capacity into increased physical activity levels (Troosters et al., 2005; Spruit et al., 2015b). Despite the benefits of pulmonary rehabilitation, there is a poor transition into higher physical activity levels following completion of the programme (Mantoani et al., 2016). COPD patients with lower physical activity levels have been suggested to have an increased risk of hospitalisation and mortality, whereas physically active COPD patients experience a reduced frequency of exacerbations (Donaire-Gonzalez et al., 2015; Garcia-Aymerich et al., 2006; Garcia-Aymerich et al., 2007; Garcia-Aymerich et al., 2009; Mercken et al., 2005; Nguyen et al., 2015). These findings, accompanied with the benefits of pulmonary rehabilitation (Studies 1-4), reiterate the importance for COPD patients to be physically active.

Understanding physical activity is complex, with suggestions that utilising step counts as a classical measure alone is insufficient as it does not account for upper body movements (Sylvia et al., 2014). Studies have utilised step counts pre-, during and post-pulmonary rehabilitation as determinants of changes in physical activity (Dallas et al., 2009; Demeyer et al., 2014; Nolan et al., 2017). Although step counts are still an important indicator of physical activity (O'Connell, OLaighin & Quinlan, 2017), research has also progressed illustrating the importance of quantifying

physical activity as time spent in various intensities of activities (Mesquita et al., 2017; van Remoortel et al., 2013). This is important given the recent shift in focus in the field of physical activity whereby more focus is being placed on time spent in a sedentary state (Physical Activity Guidelines Advisory Committee, 2018), as opposed to only monitoring whether individuals meet the physical activity guidelines of 150 min of moderate physical activity per week (Department of Health, 2011). It has recently been shown that transitions from sedentary states to light physical activity can be associated with health benefits in COPD (Mesquita et al., 2017; Physical Activity Guidelines Advisory Committee, 2018).

During onset of moderate to severe exacerbations, acute decreases in physical activity levels are common (Alahmari et al., 2014; Alahmari et al., 2016; Pitta et al., 2006), with frequent exacerbations accelerating physical activity level decline (Alahmari et al., 2014). Physical activity levels have been observed to recover within 14 days of exacerbation onset (Alahmari et al., 2014), but this hasn't always been observed as Pitta et al. (2006) still observed lower physical activity levels at 1-month post-discharge for acute exacerbation of COPD. Alahmari et al. (2016) also demonstrated that acute exacerbations can lead to larger decreases in time spent in light activity per day in frequent exacerbators. However, there are a lack of studies monitoring differences in physical activity between frequent and infrequent exacerbators when in a stable state. Further information on this would be useful in order to develop specific interventions that target physical activity in frequent exacerbators.

Studies 2, 3, and 4 have demonstrated the anti-inflammatory benefits of pulmonary rehabilitation in frequent exacerbators of COPD, as characterised by reduced fibrinogen concentrations, total leukocyte counts, and tendencies towards an increased gene expression in response to corticosteroid treatment. Being physically

active has been associated with having lower levels of systemic inflammation, as characterised by reduced fibrinogen, CRP, and total leukocytes in healthy individuals (Colbert et al., 2004; Hamer & Stamatakis, 2009; King et al., 2003). However, the benefits of being physically active have been seen to diminish following a period of physical inactivity (Fuente et al., 2005; Syu et al., 2012). In COPD, physical inactivity has been related to elevated fibrinogen and CRP concentrations (Garcia-Aymerich et al., 2009; Waschki et al., 2012; Watz et al., 2008). This is further supported by Moy et al. (2014) who found that COPD patients who have lower physical activity levels and higher levels of CRP are markedly more likely to suffer an acute exacerbation of COPD compared to those who are more physically active and have lower levels of CRP. It is important to grasp an understanding of which markers may be useful as surrogate markers of inflammation to measure in the post-pulmonary rehabilitation setting when designing future interventions to target physical activity in frequent exacerbators.

In order to inform a larger scale intervention study in the future, a pilot study was undertaken to explore physical activity and sedentary behaviour between frequent and infrequent exacerbators of COPD following pulmonary rehabilitation. Secondly, on the basis that a successful physical activity intervention would reduce levels of systemic inflammation in frequent exacerbators, potential surrogate markers that could be used as mechanistic endpoints in a future study were explored. It was hypothesised that frequent exacerbators would have lower physical activity levels and higher levels of systemic inflammation.

7.2 Methods

7.2.1 COPD patients

Forty COPD patients were approached for participation with 18 COPD patients (age 71 ± 7 years; FEV₁ pred $52 \pm 15\%$; frequent exacerbators = 9; infrequent

exacerbators = 9) successfully re-recruited >3 months following pulmonary rehabilitation.

7.2.2 Selection criteria

7.2.2.1 Inclusion criteria

Patients with a clinical diagnosis of any severity of COPD and had previously enrolled on pulmonary rehabilitation and consented to take part in Study 2.

7.2.2.2 Exclusion criteria

Patients were excluded if they displayed with any of the following: unstable ongoing cardiovascular events, other active inflammatory conditions (e.g. rheumatoid arthritis, cancer), known asthma, allergic rhinitis or other respiratory diseases (e.g. bronchiectasis, pulmonary fibrosis). Patients were also excluded if they had an inability or unwillingness to sign informed consent.

7.2.3 Recruitment

COPD patients who enrolled on to pulmonary rehabilitation and consented to take part in Study 2 were re-recruited via the Respiratory Team as a convenience sample for this pilot study. COPD patients were sent study information packs including a study information sheet and declaration of interest (DOI) form. Patients who were interested in taking part returned the DOI form in a pre-paid envelope. Interested patients were then contacted to discuss further study procedures and screen eligibility in line with the inclusion and exclusion criteria. If patients were deemed eligible to take part in the study, the first clinic visit of two was arranged at one of the following GP medical practices: Birchwood Medical Practice, Lindum Medical Practice, Nettleham Medical Practice.

7.2.4 Procedures

At the first clinic visit, COPD patients provided verbal and written consent before providing information on medical history, mainly confirming exacerbation history

and current comorbidities (presented using the Charlson Comorbidity Index (Charlson et al., 1987)), since enrolling on pulmonary rehabilitation. Patients were categorised as either a frequent (≥ 2 exacerbations in the previous 12 months) or infrequent exacerbator (< 2 exacerbations in the previous 12 months). Height and body mass measurements were then obtained before the performing of an exhaled carbon monoxide test (MicroCo Meter, CareFusion). Patients were provided with an accelerometer (wGT3X-BT, ActiGraph, Pensacola, FL, USA) and instructed to wear this around their waist for 7 days in line with previous procedures (Demeyer et al., 2014).

Following the first clinic visit, patients returned to the GP medical practice at a mutually agreed time (between 9.30am-1pm) and date (8-10 days following the first clinic visit). Accelerometers were returned to determine physical activity patterns before obtaining a blood sample in a rested state. The characteristics of physical activity included: daily step count, time spent in activity modes (sedentary, light, moderate-to-vigorous), % of time spent in positions (standing, sitting, lying), and wear time. Activity levels were determined using thresholds previously utilised in COPD patients whereby sedentary behaviour (< 1.5 METS), light physical activity (1.5 to 3 METS), and moderate-to-vigorous physical activity (> 3 METS) were used to classify each 1 minute epoch of data: sedentary behaviour (< 220); light physical activity (220-480) and moderate-to-vigorous physical activity (> 480 activity counts) (Mesquita et al., 2017). A minimum wear time of 10 hours per day (Byrom & Rowe, 2016), was required for a minimum of 5 days of measurement (Mesquita et al., 2017). Non-wear bouts were determined after 60 minutes of consecutive zero epochs across all 3 axes which is commonly utilised in physical activity studies (Byrom & Rowe, 2016).

The proposed primary clinical outcome for a future trial is daily step count. The secondary clinical outcomes were time spent in modes of activity (sedentary, light, moderate-to-vigorous), and % of time spent in positions (standing, sitting, lying). The proposed primary mechanistic endpoint for a future study is plasma fibrinogen (section 2.8.2.1). Other mechanistic outcomes included plasma CRP concentration, (section 2.8.2.2), total and differential blood leukocyte counts (section 2.8.1), and gene expression from isolated PBMC's treated with dexamethasone (MKP-1, GILZ) (section 2.8.4).

7.2.5 Statistical analysis

Due to the nature of this pilot study, analyses were treated as exploratory and mainly reported descriptively. The data will provide information on the parameters needed for a realistic sample size calculation for a future, definitive randomised controlled trial of a physical activity intervention for frequent exacerbators of COPD. Cohen's *d* effect sizes were utilised to measure differences between frequent and infrequent exacerbators for the following outcomes; daily step count, time spent in mode of activity (sedentary, light, moderate-to-vigorous), % of time spent in positions (standing, sitting, lying), fibrinogen and CRP concentrations, whole blood counts, and corticosteroid-induced gene expression (MKP-1 & GILZ, 2 hr & 6 hr). Gene expression was analysed using fold changes at 2 hr & 6 hr treatments compared to NT samples as per Study 3. Differences in measures between frequent exacerbators and infrequent exacerbators were classified as: small ($d = 0.2$), medium ($d = 0.5$), and large ($d = 0.8$) (Cohen, 1988).

7.3 Results

7.3.1 Patient characteristics

The patient characteristics are detailed in Table 7.1. Frequent exacerbators were all categorised as GOLD D (100%), with the majority of infrequent exacerbators

categorised as GOLD B (89%). The majority of frequent and infrequent exacerbators were categorised as either MRC grade 2, grade 3, or grade 4.

Table 7.1. Patient characteristics

Variable	Frequent	Infrequent
Age (years) ^a	74 ± 6	68 ± 7
% Males ^b	78%	44%
Body Mass (kg) ^a	80 ± 6	73 ± 10
GOLD grade, n (%) ^c		
A	0 (0)	1 (11)
B	0 (0)	8 (89)
C	0 (0)	0 (0)
D	9 (100)	0 (0)
FEV ₁ %predicted ^a	53 ± 16	51 ± 15
Charlson Comorbidity Index ^a	4.7 ± 1.1	3.9 ± 1.1
% Current smokers ^b	0%	33%
MRC, n (%) ^c		
0	0 (0)	1 (11)
1	0 (0)	0 (0)
2	1 (11)	3 (33)
3	3 (33)	3 (33)
4	5 (56)	2 (22)

^aData presented as mean ± SD (t-test). ^bData presented as a % of total population (chi-squared).

^cData presented as total number (% of group). Frequent, n = 9; infrequent, n = 9.

7.3.2 Physical activity levels

A decreased daily step count was observed in frequent exacerbators (mean difference -687 steps, 95% confidence interval (CI) [-856,-518], $d = 0.3$).

Differences between groups were trivial for time spent in sedentary activities per day (frequent exacerbators +7 min, 95% CI [-5,19], $d = 0.1$). Frequent exacerbators spent less time in light (-12 min, 95% CI [-15,-9], $d = 0.8$) and moderate-to-vigorous physical activity on a daily basis (-5 min, 95% CI [-6,-4], $d = 0.3$) (Figure 7.1).

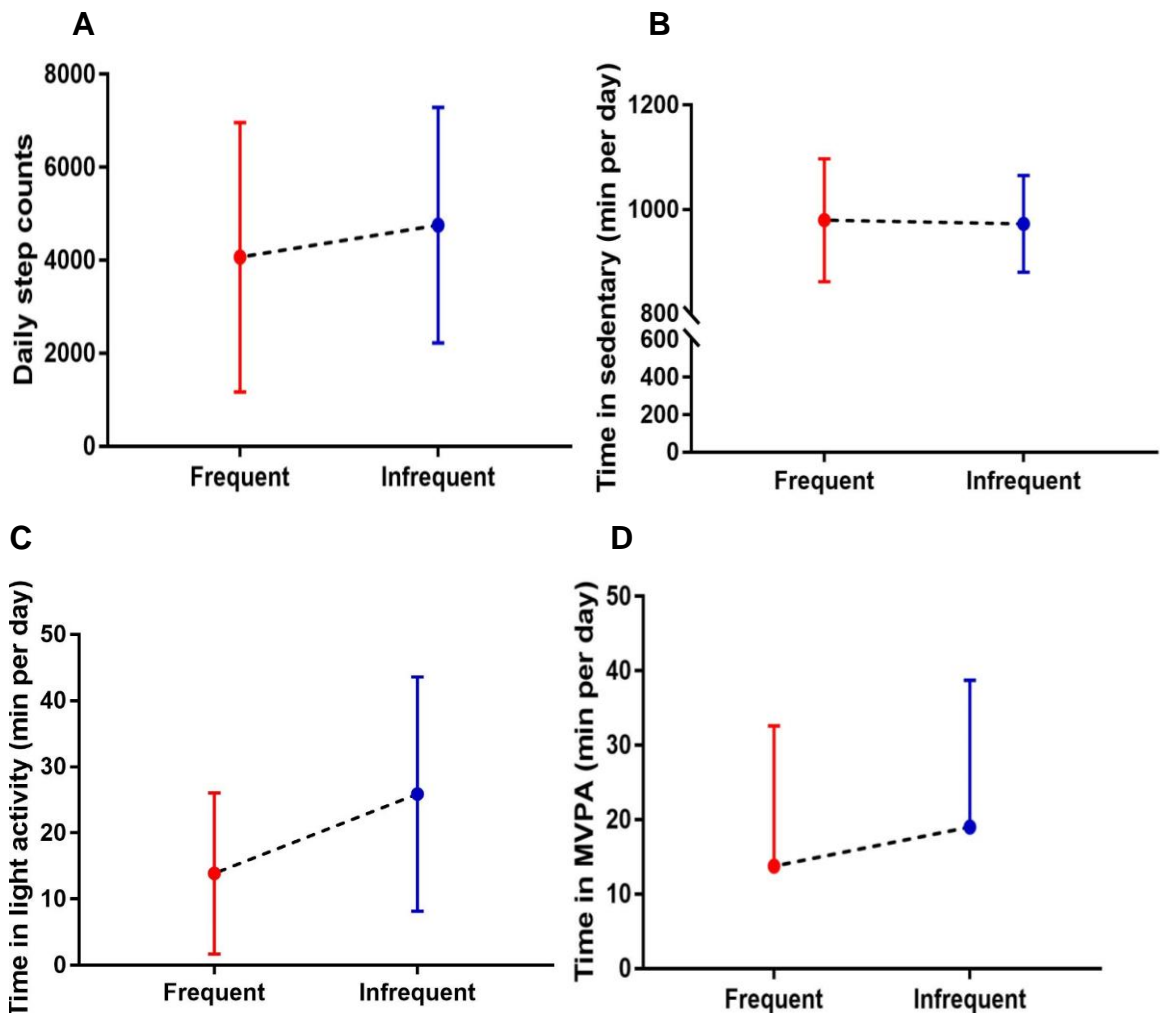


Figure 7.1. Daily step counts (A), time spent in sedentary (B), light (C) and moderate-to-vigorous intensity activity (MVPA) (D) in frequent and infrequent exacerbators.

Frequent exacerbators were observed to spend less time in standing positions (-2%, 95% CI [-3,-1], $d = 0.4$) and more time in seated positions (+4%, 95% CI [3,5], $d = 0.6$). Time spent in lying positions were observed to be lower in frequent exacerbators compared to infrequent exacerbators (-5%, 95% CI [-6,-4], $d = 0.7$)

(Figure 7.2). Frequent exacerbators recorded a lower wear time of the Actigraph (-1.5 hrs per day, 95% CI [-3,0], $d = 0.4$).

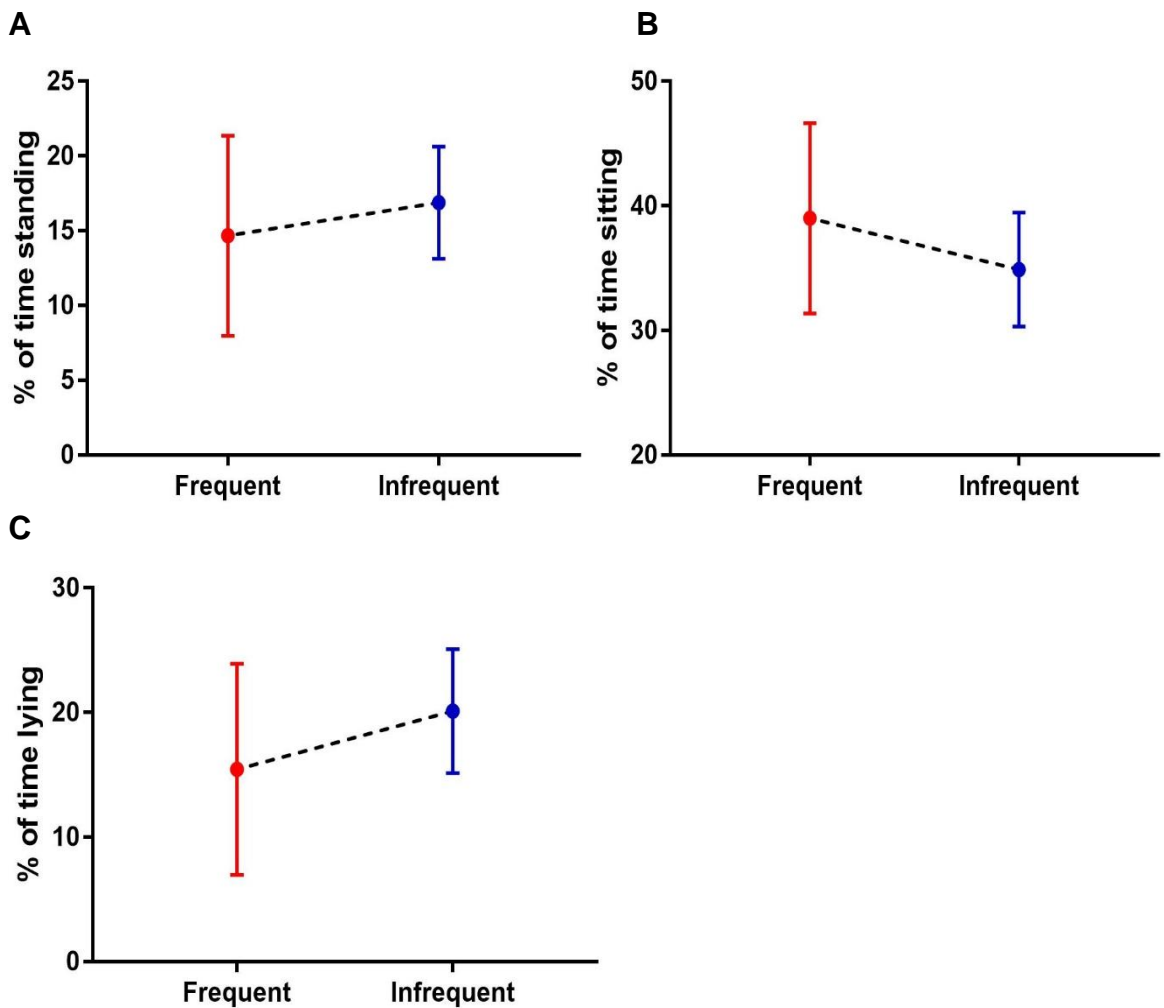


Figure 7.2. Amount of time spent in standing (A), sitting (B) and lying (C) position in frequent and infrequent exacerbators.

7.3.3 Inflammation

Fibrinogen (+19 mg/dL, 95% CI [2,36], $d = 0.3$), CRP (+3.6 mg/L, 95% CI [1.9,5.2], $d = 0.2$), and eosinophil counts (+0.02 cells per $10^9 \cdot L^{-1}$, 95% CI [0.01,0.02], $d = 0.2$) were observed to be elevated in frequent exacerbators. Total leukocyte (-1.0 cells per $10^9 \cdot L^{-1}$, 95%CI [-1.1,-0.9], $d = 0.6$), neutrophil (-0.8 cells per $10^9 \cdot L^{-1}$, 95% CI [-0.9,-0.6], $d = 0.5$), and lymphocyte counts (-0.3 cells per $10^9 \cdot L^{-1}$, 95% CI [-0.4,-0.2], $d = 0.7$) were seen to be lower in frequent exacerbators. The differences between

frequent (-0.1, 95% CI [-0.2,-0.1], $d = 0.1$) and infrequent exacerbators for neutrophil/lymphocyte ratio were trivial (Table 7.2).

Table 7.2. Inflammatory parameters between frequent and infrequent exacerbators

Variable	Frequent	Infrequent	Cohen's d
Fibrinogen (mg/dL) ^a	783 ± 58	764 ± 96	0.3
C-Reactive Protein (mg/L) ^b	7.3 (3.3-8.0)	3.4 (2.3-4.1)	0.2
Total Leukocytes (10 ⁹ ·L ⁻¹) ^a	6.6 ± 1.8	7.6 ± 1.5	0.6
Eosinophils (10 ⁹ ·L ⁻¹) ^a	0.2 ± 0.1	0.2 ± 0.1	0.2
Neutrophils (10 ⁹ ·L ⁻¹) ^a	4.1 ± 1.6	4.8 ± 1.3	0.5
Lymphocytes (10 ⁹ ·L ⁻¹) ^a	1.5 ± 0.3	1.8 ± 0.5	0.7
Neutrophil/Lymphocytes ratio ^a	2.8 ± 1.0	2.9 ± 1.1	0.1

^aData presented as mean ± SD. ^bData presented as a median (25th percentile – 75th percentile). Frequent, n = 9; infrequent, n = 9.

7.3.4 Gene expression

Differences in MKP-1 expression following dexamethasone treatment for 2 hr between frequent (+0.1 fold, 95% CI [0.12,0.14], $d = 0.1$) and infrequent exacerbators were observed to be trivial, but 2 hr treatment on GILZ expression suggested a lower response in frequent exacerbators (-1.5 fold, 95% CI [-1.9,-1.1], $d = 0.8$) (Figure 7.3).

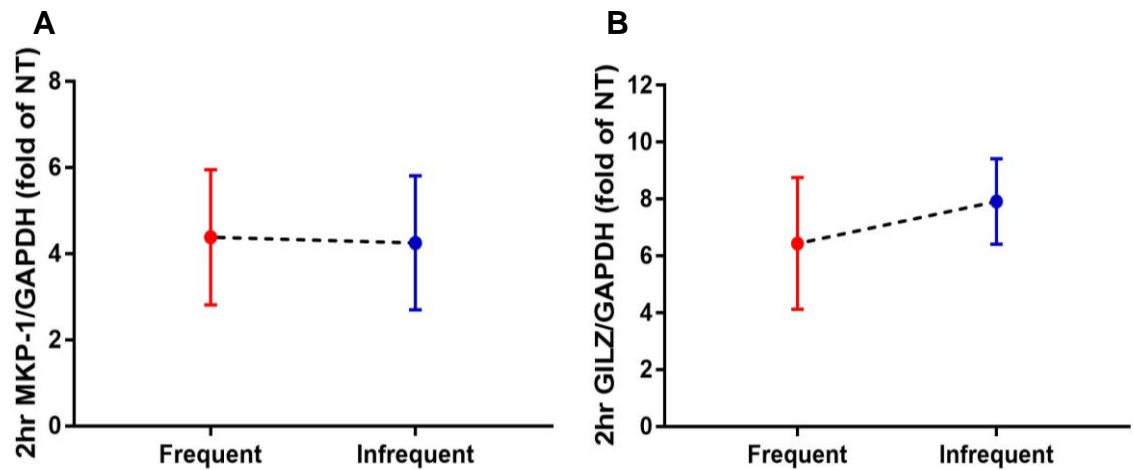


Figure 7.3. Fold changes in MKP-1 (A) and GILZ (B) gene expression from NT in isolated PBMC's following 2 hr of 1µM dexamethasone treatment in frequent and infrequent exacerbators of COPD.

Trivial effects were observed with MKP-1 expression following 6 hr of dexamethasone treatment (frequent exacerbators -0.3 fold, 95% CI [-0.4,-0.1], $d = 0.1$) between frequent and infrequent exacerbators, whereas GILZ expression was lower in response to 6 hr dexamethasone treatment in frequent exacerbators (-1.5 fold, 95% CI [-1.7,-1.3], $d = 0.5$) (Figure 7.4).

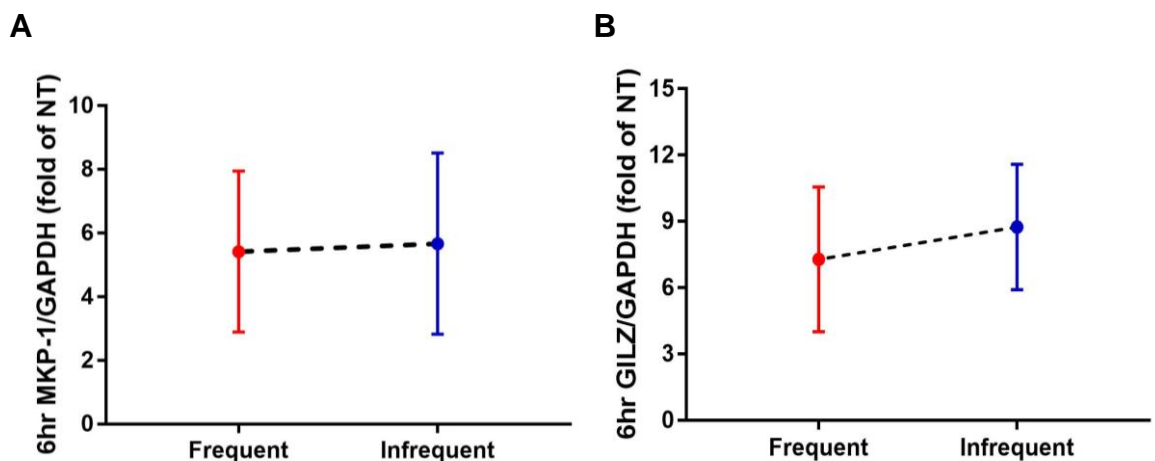


Figure 7.4. Fold changes in MKP-1 (A) and GILZ (B) gene expression from NT in isolated PBMC's following 6 hr of 1µM dexamethasone treatment in frequent and infrequent exacerbators of COPD.

7.4 Discussion

The main findings from this pilot study suggest that frequent exacerbators record fewer daily steps and spend less time in light and moderate-to-vigorous intensity

activities. The current findings also suggested that frequent exacerbators spend less time in standing positions and more time in sitting positions. Frequent exacerbators also provided indications of having heightened levels of fibrinogen and CRP, and lower anti-inflammatory gene in response to corticosteroid treatment.

It is well established that COPD patients are less physically active (Arne et al., 2011; Pitta et al., 2005; Vorrink et al., 2011), and that exacerbations can lead to acute decreases in physical activity levels (Alahmari et al., 2014; Alahmari et al., 2016; Pitta et al., 2006), with recovery of activity levels not always observed following acute exacerbations (Pitta et al., 2006). However, it is unclear whether certain phenotypes, for example frequent exacerbators, are more susceptible to physical inactivity. This study provides preliminary evidence towards suggesting that frequent exacerbators, when stable, may spend more time in sedentary states as characterised by reduced steps, less time in light and moderate-to-vigorous physical activities, and spending more time sitting. Frequent exacerbators were observed on average to make 687 less steps per day compared to infrequent exacerbators which falls within the threshold for the proposed MCID for step counts in COPD (Demeyer et al., 2016). The step counts observed in the current study are comparable to that cited in previous literature assessing physical activity in COPD patients (Demeyer et al., 2014; Demeyer et al., 2016). This evidence provides support to previous research which suggested, despite a lack of statistical significance, that frequent exacerbators spend more time in sedentary states and less time in higher physical activities (Valido et al., 2014). There is also evidence suggesting that frequent exacerbators experience a faster decline in physical activity over time (Alahmari et al., 2014; Donaldson et al., 2005b). Possible explanations for the physical inactivity in frequent exacerbators may be the perception of exacerbating symptoms with exertion (Troosters et al., 2013), or an

accumulation of the acute effects of each exacerbation on physical activity level (Alahmari et al., 2014). It is important to address physical activity levels in frequent exacerbators, given that being physically active has been associated with a reduction in hospitalisations and mortality (Donaire-Gonzalez et al., 2015; Garcia-Aymerich et al., 2006; 2007; 2009; Mercken et al., 2005; Nguyen et al., 2015), and future interventions should target ways of addressing this issue. A starting point, as previously suggested by Mesquita et al. (2017), would be to target sedentary behaviour in favour of light activities, which may be more easily achievable than inducing increases in moderate activities (Spruit et al., 2015b). This is supported by a study suggesting that a greater quantity of low-intensity physical activity is effective at reducing the risk of COPD hospitalisation (Donaire-Gonzalez et al., 2015). However, it is unclear whether it is structured physical activity (i.e. exercise) programmes or general physical activity behaviours that have greatest influence on exacerbation frequency as exercise maintenance programmes post-pulmonary rehabilitation have also been shown to reduce exacerbation risk (Jenkins et al., 2018).

Another important factor associated with physical activity is the relationship with reduced levels of inflammation in both healthy (Colbert et al., 2004; Hamer & Stamatakis, 2009; King et al., 2003), and COPD populations (Gimeno-Santos et al., 2014). This study suggests that fibrinogen and CRP which are known biomarkers of exacerbations (Donaldson et al., 2005a; Hurst et al., 2010; Wedzicha, Mackay & Singh, 2013), are worthwhile markers to assess in the context of physical activity in frequent exacerbators due to the scope for improvement in inflammation with physical activity interventions in this population. The use of these markers has also been supported in previous research suggesting physically active COPD patients have lower levels of CRP (Kantorowski et al., 2018; Moy et al., 2014). The visually

elevated levels of fibrinogen and CRP in the frequent exacerbators are in line with pre-pulmonary rehabilitation observations in Study 2. However, total leukocyte, neutrophil, and lymphocyte counts were observed to be lower in frequent exacerbators which disagrees with observations in Study 2 where these cell subsets were visually elevated in frequent exacerbators pre-pulmonary rehabilitation. Given the post-pulmonary rehabilitation population utilised in this study, it is important to note the reductions observed in total leukocyte and neutrophil counts in frequent exacerbators following pulmonary rehabilitation (Study 2) may have an influence on the findings of the current study. In other words, markers that are sensitive to changes with pulmonary rehabilitation may not relate to all measures of physical activity. Also, pulmonary rehabilitation may act to restore perturbations in inflammatory measures between frequent and infrequent exacerbators and that greater physical activity levels are required to see further changes in inflammatory measures. This reiterates the importance of the requirement for larger scale trials utilising proposed inflammatory markers of frequent exacerbators.

Study 3 was the first study to assess changes in gene expression in response to corticosteroid treatment in frequent and infrequent exacerbators of COPD undertaking pulmonary rehabilitation. The current study aimed to further assess the differences between frequent and infrequent exacerbators at rest to see how the groups compare following pulmonary rehabilitation. The current study suggested that frequent exacerbators had a poorer response to corticosteroid treatment as well as poorer physical activity levels. Interestingly, this was observed with GILZ expression whereas Study 3 only observed changes or differences between groups with MKP-1 expression. These results suggest that physical activity levels and exacerbation history may play a role in the ability of corticosteroids to amplify anti-

inflammatory GILZ gene expression. Further research is warranted building on the current findings, and that of Study 3, to see whether physical activity levels may be an independent determinant in the effectiveness of corticosteroid treatment in COPD and determine whether MKP-1 and/or GILZ are key pathways to explore moving forwards.

The findings of this study may be used to inform a sample size calculation and decision over the primary outcome of a future intervention study targeting physical activity behaviours in frequent exacerbators. Based on the available data, to detect a change of 687 steps in frequent exacerbators a sample size of 247 participants would be required to have 80% power at the 5% level of significance. Based on reversing the differences seen in frequent exacerbators in other measures of this study, the following sample sizes would be required: 26 participants for time spent in light activity, 239 participants for time spent in moderate-to-vigorous activity, 128 participants for time spent standing, 123 participants for time spent sitting.

When interpreting the findings of this study, it is important to note the limitations. This study was a pilot study assessing a small population of COPD patients. Inflammatory parameters in COPD patients have been seen to vary significantly between patients (Sapey et al., 2008), making it difficult to draw comparisons with small sample sizes. It is also important to note that the cohort of patients recruited had recently completed pulmonary rehabilitation (<18 months) and it cannot be excluded whether the anti-inflammatory benefits observed in Study 2 were still maintained whereby pulmonary rehabilitation appeared to alleviate differences in inflammation between frequent and infrequent exacerbators. Also, the location of the device may have influenced the data presented in this study as the wearing of the accelerometer on the thigh has been proposed to be the optimal location for analysing sedentary behaviours (Byrom & Rowe, 2016). Finally, this study did not

exclude smokers and the impact of this on physical activity levels and inflammation must be considered when interpreting the findings.

In conclusion, this pilot study suggests that frequent exacerbators are less physically active than infrequent exacerbators. Frequent exacerbators were suggested to have a poorer response to corticosteroid treatment potentially relating physical activity levels to the effectiveness of corticosteroid treatment. It is unclear whether physical inactivity is a cause of the frequent exacerbator phenotype or whether it is an effect of recurrent exacerbations. Future trials should attempt to establish causal relationships between the frequent exacerbator phenotype and physical activity. It would be worthwhile for future studies to clarify the role of inflammation in mediating this relationship, the current study provides some surrogate markers that could be used for that purpose.

8 Chapter EIGHT

General Discussion

The series of studies presented in this thesis (Studies 1-5) were designed to assess the frequent exacerbator phenotype of COPD in the context of pulmonary rehabilitation. The main focus of this thesis was to see whether the frequent exacerbator phenotype responds differently to pulmonary rehabilitation when compared to infrequent exacerbators, in terms of clinical outcomes at a patient level and one of the key underlying biological mechanisms of this frequent exacerbator phenotype, systemic inflammation (Bhowmik et al., 2000; Donaldson et al., 2005a; Perera et al., 2007).

Firstly, this thesis examined whether the frequent exacerbator of COPD phenotype had any impact on the completion rates and benefits of pulmonary rehabilitation (Study 1). As inflammation is a key therapeutic target of the frequent exacerbator phenotype, the following studies assessed the inflammatory responses to chronic exercise in the form of pulmonary rehabilitation (Studies 2 and 3). The studies were designed to see whether pulmonary rehabilitation could be effective in reducing resting inflammation in frequent and infrequent exacerbators and to see whether changes in immune profiles were similar in both groups. Previously identified elevated inflammatory mediators in frequent exacerbators were selected for analysis in the form of fibrinogen, CRP, total leukocytes and neutrophils (Study 2; Donaldson et al., 2005a; Hurst et al., 2010; Wedzicha et al., 2013). Novel measures of inflammation were also investigated in these studies, specifically whether chronic exercise modified measures of blood neutrophil activation or subsets (Study 2) and expression of corticosteroid inducible genes (Study 3). Inflammatory responses to exercise were assessed in two different contexts in this thesis. The studies of

chronic exercise were followed up with assessment of acute inflammatory responses to exercise in frequent and infrequent exacerbators (Study 4), given that acute exercise has previously been proposed to trigger an inflammatory response in COPD patients (van Helvoort et al., 2006). The assessment of acute responses were split into two sub-studies with the aim of assessing inflammatory responses to exercise in both an 'untrained' (beginning of rehabilitation) and 'trained' (end of rehabilitation) state. Finally, a pilot study of follow-up assessments of physical activity levels and inflammation were undertaken in a subset of frequent and infrequent exacerbators post-pulmonary rehabilitation to see whether future physical activity interventions in the frequent exacerbator phenotype would be worthwhile (Study 5).

8.1 Clinical outcomes of pulmonary rehabilitation

8.1.1 Completion rates

Despite the benefits of pulmonary rehabilitation in increasing exercise capacity and improving quality of life (McCarthy et al., 2015), there is still a major issue with completion rates of pulmonary rehabilitation (Steiner et al., 2018). Factors such as depression, smoking status, frailty, and social deprivation have been linked to poor completion of pulmonary rehabilitation (Hayton et al., 2013; Keating, Lee & Holland, 2011; Maddocks et al., 2016; Steiner et al., 2017b). However, it was unclear whether a recognised phenotype such as frequent exacerbators were at a higher risk for poor completion given that exacerbations are a risk factor for non-completion (Fischer et al., 2009). The first experimental study detailed in Study 1 highlighted the poorer completion rates in frequent exacerbators when compared to infrequent exacerbators. This provides evidence towards the proposed notion that exacerbations are a prominent cause of poor completion of pulmonary rehabilitation (Fischer et al., 2009; Hayton et al., 2013; Keating, Lee & Holland,

2011). Although it is plausible to think that this is the case in a clinical setting, there is a lack of evidence demonstrating that this is conclusively the case. Completion rates have been a long-serving issue for clinicians when delivering pulmonary rehabilitation and have been highlighted by the UK National COPD Pulmonary Rehabilitation Audit (Steiner et al., 2018). For the first time, Study 1 highlights the potential use for phenotyping COPD patients to identify potentially 'at risk' populations for poor completion of pulmonary rehabilitation.

In theory, it could be expected that COPD patients who have 2 or more exacerbations within 12 months (frequent exacerbators) are more at risk of non-completion. Especially given that a pulmonary rehabilitation course takes place over a sizeable period of the year, particularly if this spanned the winter period or if one considers the time lapses between initial and final assessments (usually 1 week to 1 month before or after pulmonary rehabilitation) meaning that the window of pulmonary rehabilitation treatment could last up to 4 months. Clearly, this is a significant issue for patients who experience at least 2 exacerbations in a 12 month period.

Previously, it has been suggested that frequent exacerbators are a rare phenotype of COPD (Han et al., 2017), but Study 1 shows for the first time that this may not necessarily be the case when assessing COPD patients presenting to pulmonary rehabilitation programmes. In the cohort recruited to this study, 59% of patients were classified as frequent exacerbators. With poor completion rates being a worldwide issue with pulmonary rehabilitation, and a specific target of the ongoing UK National COPD Pulmonary Rehabilitation Audit (Steiner et al., 2018), it is possible that the message arising from this study is that the 'elevated' presence of frequent exacerbators may be playing a significant role. It is important to consider that the elevated presence of frequent exacerbators in pulmonary rehabilitation

maybe due to underlying reasons for referral (e.g. recent exacerbation) (Bolton et al., 2013). Nevertheless, regardless of whether frequent exacerbators are 'rare' or not, this phenotype represents a large social and economic burden in COPD (Wedzicha et al., 2013), including poorer completion rates of pulmonary rehabilitation presented in Study 1.

COPD patients who present to pulmonary rehabilitation with poorer baseline measurements have been observed to have larger improvements in outcomes upon completion (Sprit et al., 2015a). It was plausible to hypothesise that frequent exacerbators may stand to experience larger improvements in clinical outcomes when compared to infrequent exacerbators as it has been suggested previously that frequent exacerbators demonstrated poorer baseline measures (Valido et al., 2014). The assessment of frequent and infrequent exacerbators who completed pulmonary rehabilitation demonstrated statistically and clinically significant improvements in clinical outcomes. However, no clinical or significant differences in baseline measures were seen between frequent and infrequent exacerbators suggesting responses to pulmonary rehabilitation were similar in magnitude. These conflicting findings require further assessment in future research. The beneficial responses observed reiterates the importance of making sure that pulmonary rehabilitation is inclusive to all COPD patients, especially as both phenotypes stand to benefit from the intervention.

Although both frequent and infrequent exacerbators benefit from pulmonary rehabilitation upon completion, this still leaves a significantly unanswered question, what about the COPD patients who do not complete? Are these patients more impaired in terms of exercise capacity and psychological status making the prospect of pulmonary rehabilitation more daunting? Is pulmonary rehabilitation perceived to be of a lack of benefit to them, hence poor completion? The

assessment of this demographic of patients requires further research to enable the tailoring of interventions to help support these COPD patients towards pulmonary rehabilitation completion as completion rates may stem from factors beyond that of exacerbation history. With further evidence, steps can be made to specifically assess what interventions can be undertaken to help support frequent exacerbators through pulmonary rehabilitation. Firstly, efforts need to be made to establish the underlying causes of poor completion in frequent exacerbators.

Assessment of the data collected in Study 1 in terms of baseline measures, found that the exercise capacity for those that completed (mean difference; ISWT: frequent +99m, infrequent +82m; ESWT: frequent +120m, infrequent +126m) pulmonary rehabilitation was higher at baseline compared to those that failed to complete by clinically significant margins (Figure 8.1A & Figure 8.1B). Baseline total CRQ scores were seen to be poorer in frequent exacerbators who did not complete (-3.1 points) whereas baseline scores appeared to be higher in infrequent exacerbators who did not complete pulmonary rehabilitation compared to those that did (+0.6 points) (Figure 8.1C). Further breakdown unveiled that non-completers reported poorer scores on the fatigue domain of the CRQ (frequent -0.8 points, infrequent -0.6 points). Similar observations were made with anxiety scores as non-completing frequent exacerbators had higher baseline anxiety compared to frequent exacerbator completers (+2.5 points) whereas non-completing infrequent exacerbators had lower anxiety compared to infrequent exacerbator completers (-2.7 points) (Figure 8.1 D). Both non-completing frequent (+1.2 points) and infrequent exacerbators (+0.9 points) had higher baseline levels of depression compared to completing frequent and infrequent exacerbators (Figure 8.1E). These data warrant further investigations of the characteristics of non-completers as these patients may have poorer baseline exercise capacity and poorer control over their

condition which maybe increasing the risk of non-completion. Placing focus on this may identify a population who stand to potentially have more scope to benefit from pulmonary rehabilitation further reiterating the need to intervene with these patients.

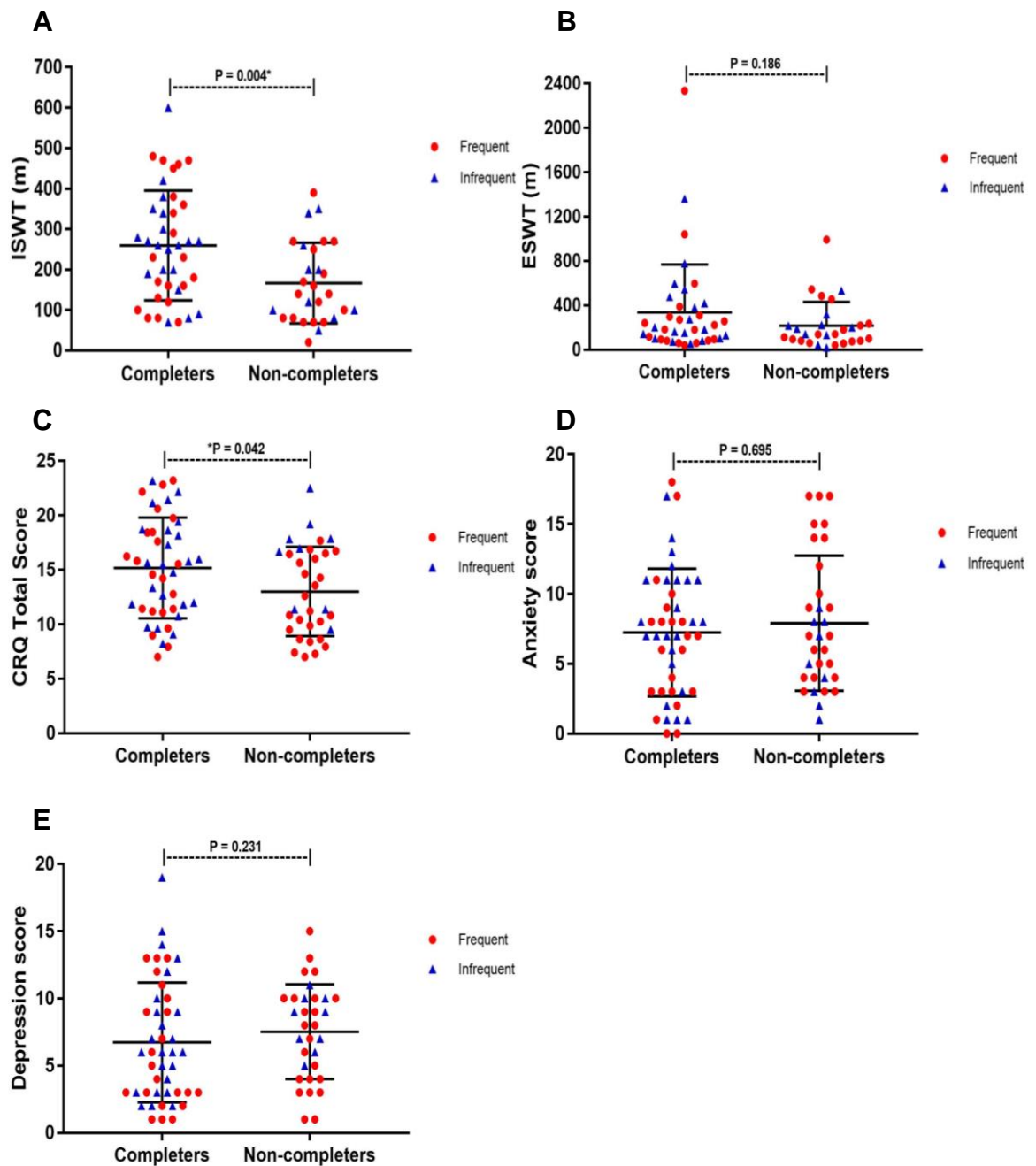


Figure 8.1. Baseline clinical measures for ISWT (A), ESWT (B), total CRQ score (C), Anxiety score (D), and Depression score (E) in frequent and infrequent exacerbators who completed compared to frequent and infrequent exacerbators who did not complete. Statistics analysed using a Mann-Whitney U test to compare differences between completers and non-completers. *Significant difference between groups ($p < 0.05$). Bars represent mean values \pm SD.

A notable limitation arising from Study 1 is the lack of information surrounding reasons for drop out of pulmonary rehabilitation. This provides a lack of clarity in the underlying reasons for poor completion in the frequent exacerbator phenotype whereby factors not independently related to this phenotype, such as transport and lack of social support, have been previously associated (Hayton et al., 2013; Keating, Lee & Holland, 2011). This thesis has adopted a different approach of looking at a specific phenotype of COPD which may incorporate some factors that have previously been shown to relate to poor completion (e.g. smoking status and depression) (Hayton et al., 2013; Keating, Lee & Holland, 2011). Study 1 showed patients were matched for smoking status and HADS at baseline but the effects of other known factors such as frailty and social deprivation cannot be dismissed (Maddocks et al., 2016; Steiner et al., 2017b). Future research, which could be undertaken as part of a clinical audit, should establish the factors relating to poor completion in frequent exacerbators before considering adopting the phenotyping approach in the context of pulmonary rehabilitation.

The big question that would be left unanswered, and is certainly beyond the scope of this thesis, would be how to get more frequent exacerbators of COPD to complete pulmonary rehabilitation. Completion of pulmonary rehabilitation is currently a hot topic in the COPD community with a magnitude of work left to undertake tackling this issue. It would be speculative to make specific recommendations in this area, but the available evidence from this thesis does at least suggest that the frequent exacerbator phenotype are a prevalent, specific group of patients where a consideration of interventions to support completion of pulmonary rehabilitation is needed.

8.1.2 Maintenance of pulmonary rehabilitation

Having established the benefits of pulmonary rehabilitation in terms of clinical outcomes for both frequent and infrequent exacerbators, Study 5 concluded the experimental aspect of this thesis by following up a subset of frequent and infrequent exacerbators post-pulmonary rehabilitation. One of the main concerns surrounding pulmonary rehabilitation is the lack of effective lifestyle change (e.g. increased physical activity levels) in subsets of COPD patients upon completion (Mesquita et al., 2017). Maintenance exercise programmes have been shown to be beneficial for patients with COPD in maintaining and sometimes even enhancing the benefits obtained from the initial course (Jenkins et al., 2018). However, these programmes are scarce and there is a distinct general lack of feasible options for COPD patients following pulmonary rehabilitation. Furthermore, evidence has also suggested that the intensity of physical activity is important to consider when looking at the susceptibility to exacerbation (Donaire-Gonzalez et al., 2015). Given the newly established findings in the context of this thesis, it was interesting to assess physical activity levels in subsets of patients following pulmonary rehabilitation.

There is limited evidence on physical activity in frequent exacerbators, but previous evidence has suggested that frequent exacerbators may be less active compared to infrequent exacerbators (Valido et al., 2014). Furthermore, frequent exacerbators have been seen to have faster declines in physical activity levels (Alahmari et al., 2014). However, there is still a lack of clarity around physical activity levels in frequent exacerbators. Study 5 aimed to assess physical activity characteristics in frequent and infrequent exacerbators of COPD following pulmonary rehabilitation.

Although Study 5 was only a pilot study to inform further larger scale trials, there were early indications that physical activity levels were lower in frequent

exacerbators following pulmonary rehabilitation. The most notable observations were the lower recorded daily step counts and the lesser amount of time spent in lower intensity physical activity in frequent exacerbators. This has been raised as a potentially important target for reducing incidence of exacerbations in COPD by transitioning sedentary activity into light activity, rather than placing too much emphasis on trying to transition straight into higher intensity activities (Donaire-Gonzalez et al., 2015; Mesquita et al., 2017). Placing focus on transitioning into higher intensities could be detrimental from the psychological aspect of the patient whereby heightened levels of activity may be perceived to exacerbate symptoms which could trigger a cascade of events back to physical inactivity (Fischer et al., 2007; Harris, Hayter & Allender, 2008; Taylor et al., 2007; Young et al., 1999). Therefore, placing focus on lighter physical activities in the initial phase, may be more beneficial given its ability to reduce exacerbations of COPD (Donaire-Gonzalez et al., 2015), especially in a population such as frequent exacerbators who are more susceptible to exacerbations.

COPD patients with lower physical activity levels have been suggested to have heightened levels of systemic inflammation (Garcia-Aymerich et al., 2009; Waschki et al., 2012; Watz et al., 2008). However, despite the differences in physical activity levels between frequent and infrequent exacerbators there were no definitive differences in inflammation. Given the pilot nature of this study combined with the variable inter-patient inflammation (Sapey et al., 2008), further larger scale trials are warranted. However, this pilot study provided indications on suitable markers for use, placing emphasis on the utility of fibrinogen as a useful biomarker in frequent exacerbators. CRP was highly variable, in agreement with previous research (Aksu et al., 2013), and this should be considered when designing future trials.

This pilot study provided useful information on physical activity in the frequent exacerbator phenotype following pulmonary rehabilitation which can be used to design future research trials monitoring physical activity in this phenotype with the aim of developing targeted physical activity interventions to maintain the benefits of pulmonary rehabilitation. If this research study was to be undertaken again, a larger sample size would be recruited utilising different inclusion/exclusion criteria. For example, enlarge the potential recruitment pool by opening recruitment to all COPD patients and not just those who took part in Study 2. It would also be insightful to have an inflammatory profile timeline with assessment of physical activity and inflammation at pre-rehabilitation, post-rehabilitation and then at a designated follow-up time point. This would allow for stronger correlations between the effects of pulmonary rehabilitation and physical activity on inflammation to see whether maintenance or enhancement of physical activity levels following completion of pulmonary rehabilitation translates to modulated inflammation compared to those who have decline in physical activity levels.

8.2 Inflammation

8.2.1 Inflammatory biomarkers of exacerbations and frequent exacerbators

It has been established that several markers of systemic inflammation increase in response to exacerbations (Hurst et al., 2010). Research has also suggested that the frequent exacerbator phenotype presents with higher levels of fibrinogen in a stable state compared to infrequent exacerbators (Donaldson et al., 2005a). It is believed that plasma fibrinogen levels are elevated in COPD due to the systemic inflammatory nature of the disease triggered by persistent elevated IL-6 levels (Wedzicha et al., 2000). In frequent exacerbators, elevated IL-6 and CRP levels are observed in recovery periods following exacerbation contributing to persistent post-exacerbation inflammation, which may in part explain the higher baseline

inflammation (Perrera et al., 2007; Wedzicha et al., 2013). An inability to resolve inflammation following exacerbation has been linked to clustering of events which may explain why frequent exacerbators are more susceptible to exacerbations (Wedzicha et al., 2013). In Studies 2 & 3, baseline comparisons were made between frequent and infrequent exacerbators for markers of inflammation.

8.2.2 Baseline differences

8.2.2.1 Fibrinogen

Fibrinogen has been heavily implicated in the frequent exacerbator phenotype and is regarded as an important biomarker for exacerbations (Hurst et al., 2010). In Study 2, frequent exacerbators had non-significant, elevations in fibrinogen levels compared to infrequent exacerbators. This agrees with previous research which demonstrated that fibrinogen is elevated in frequent exacerbators (Donaldson et al., 2005a; Hurst et al., 2010). The findings in Study 2 provided further evidence of the usefulness of fibrinogen as a marker in frequent exacerbators.

8.2.2.2 C-reactive protein

CRP has also been identified as a biomarker for exacerbations (Thomsen et al., 2013), and is elevated in frequent exacerbators (Perera et al., 2007). The data presented in Study 2 provided further suggestions that CRP might be elevated in the frequent exacerbator phenotype, however variability was large with the use of this marker agreeing with previous findings (Aksu et al., 2013). The variability demonstrated when assessing CRP may suggest that this marker is not the most suitable for detecting patients more likely to exacerbate, or patients who are frequent exacerbators. Whilst CRP may still prove useful as a marker for assessment in frequent exacerbators, caution should be urged with the sensitivity of CRP to change.

8.2.2.3 Total leukocyte and neutrophil counts

Total leukocyte and neutrophil counts have been commonly observed to be elevated during exacerbations as well as in a stable state in frequent exacerbators (Hurst et al., 2010). Study 2 provided further evidence that elevated total leukocyte and neutrophil counts maybe implicated in the frequent exacerbator phenotype. Therefore, total leukocyte and neutrophil counts are worthy markers to assess in frequent exacerbators. However, this was not repeated in Study 5 which may suggest they are not useful in all settings (e.g. physical activity) as fibrinogen.

8.2.2.4 Neutrophil activation markers

It has been acknowledged that neutrophil counts provide little information on neutrophil function, whereby the latter may be of more importance when assessing inflammation (Oudijk et al., 2005). In COPD, neutrophil activation markers such as CD11b & CD66b have been seen to be upregulated whilst CD62L has been shown to be downregulated in comparison to healthy subjects (Fortunati et al., 2009). However, no comparisons of neutrophil activation, or neutrophil phenotypes have been made in frequent and infrequent exacerbators. The data presented in Study 2 suggested that CD11b and CD66b expression were similar between frequent and infrequent exacerbators. Frequent exacerbators did show a tendency towards decreased neutrophil CD62L expression at rest, which is indicative of an enhanced neutrophil activation (Takahashi et al., 2013). These markers were assessed on small subsets of patients and it would be insightful to undertake further assessments of these markers, in particular neutrophil CD62L.

For the first time in COPD, Study 2 undertook phenotyping of neutrophils following the recent identification of four neutrophil subsets (mature, immature, suppressive, progenitor) (Cortjens et al., 2017). Frequent exacerbators showed a tendency towards having a lower percentage of mature neutrophils and a higher percentage

of suppressive neutrophils compared to infrequent exacerbators. This would suggest that frequent exacerbators have impaired neutrophil phenotype as characterised by having larger percentages of neutrophils which suppress functional capabilities. Given the promising findings presented, it would be worthwhile adopting this neutrophil phenotyping approach further in the context of future research in COPD.

8.2.2.5 Corticosteroid responses

ICS are a commonly used anti-inflammatory pharmacological therapy in COPD (Jen, Rennard & Sin, 2012). However, the effectiveness of ICS has been called into question (Ernst, Saad & Suissa, 2015), due to a developed insensitivity in COPD patients (Lo Tam Loi et al., 2013). Study 3 was the first study to compare the responses of corticosteroid inducible genes following treatment with dexamethasone in frequent and infrequent exacerbators. The results suggested that frequent exacerbators may have a poorer response to corticosteroid treatment compared to infrequent exacerbators, which was further observed in Study 5. This would imply that corticosteroids are less effective in frequent exacerbators and may in part explain the inability to suppress inflammation and exacerbations in this phenotype. These promising findings should be explored further to confirm the findings reported in Studies 3 & 5 and develop an understanding of the mechanisms for a reduced response to corticosteroid treatment.

8.2.3 Inflammatory responses to exercise

Having highlighted the benefits of pulmonary rehabilitation in both frequent and infrequent exacerbators, in terms of commonly used clinical outcomes, this thesis began to address an underexplored and important area, exercise immunology in COPD. It is well established that chronic inflammation is a central component underpinning the pathophysiology of COPD (Rovina, Koutsoukou & Koulouris,

2013; Stockley, Mannino & Barnes, 2009). However, there are currently a lack of treatments aimed at controlling systemic inflammation in COPD (Santos et al., 2016).

The concept of exercise immunology in clinical populations is very much in its infancy. The anti- and pro-inflammatory effects of exercise in healthy populations have led to suggestions that exercise can be used as a treatment to ameliorate the symptoms of chronic conditions (Gleeson et al., 2011). These findings, combined with the observations of reduced exacerbations with pulmonary rehabilitation (Moore et al., 2016), and enhanced effects with continued maintenance exercise (Jenkins et al., 2018), provided the rationale for exploring the effects of exercise on inflammatory parameters. However, it is important to reiterate that pulmonary rehabilitation is not specifically aimed at reducing inflammation in COPD (Evans & Steiner, 2018). Following the poorer completion rates in frequent exacerbators (Study 1), and the identification of heightened inflammation in this phenotype (Bhowmik et al., 2000; Donaldson et al., 2005a; Perera et al., 2007), it was important to assess inflammatory processes in frequent exacerbators and whether pulmonary rehabilitation can have similar, or more beneficial, effects in this phenotype. This follows a call for a better understanding of the potential unknown mechanistic benefits of pulmonary rehabilitation given the limited available evidence (Jenkins, Holden & Jones, 2018).

8.2.3.1 Fibrinogen

Studies 2 & 4 were the first studies to utilise fibrinogen in the context of exercise immunology in COPD. Study 2 showed that pulmonary rehabilitation reduced the concentration of fibrinogen. For the first time, this provides the foundations for demonstrating anti-inflammatory effects of pulmonary rehabilitation in COPD as characterised by a reduction in the resting concentration of fibrinogen. Based on

evidence presented in Study 1, it would appear that in frequent exacerbators who complete pulmonary rehabilitation, reductions in fibrinogen concentrations occur alongside improvements in traditional clinical outcomes (e.g. exercise capacity). Study 4 progressed the utilisation of fibrinogen by examining the responses to acute exercise. Fibrinogen has been previously observed to be elevated post-exercise in a healthy population (Montgomery et al., 1996). Study 4 suggested that acute exercise does not exacerbate fibrinogen concentrations in patients with COPD regardless of exercise training. However, the acute exercise undertaken in previous studies of healthy populations was strenuous and prolonged (Montgomery et al., 1996), suggesting that higher exercise intensities and durations are required to trigger elevations in fibrinogen. The findings presented in Study 2 warrant further investigation to provide the mechanistic effects of exercise training on reduced fibrinogen concentration and whether these reductions correspond to a reduced incidence of exacerbations. Currently, the mechanisms underpinning a reduced fibrinogen concentration following exercise training are poorly understood. Nevertheless, the previously suggested ability of IL-6 to mediate fibrinogen concentrations (Gabay & Kushner, 1999; Mackiewicz et al., 1991), may in part explain the current findings as IL-6 levels have been shown to be decreased following regular exercise training (Fischer, 2006). However, IL-6 was not measured in this thesis and comparisons could not be drawn between IL-6 and fibrinogen. Another proposed mechanism is a change in adiposity with exercise training (Bodary et al., 2003). Adiposity itself is associated with inflammation, and a reduction in adiposity with exercise may lead to a reduction in fibrinogen levels with further acute phase reactants also reduced (Panagiotakos et al., 2005). The findings in Study 4 also require further research to assess changes in fibrinogen concentration in response to acute bouts of exercise varying in

duration and intensity, particularly with the interest in the use of interval exercise programmes in COPD.

8.2.3.2 C-reactive protein

In the context of exercise immunology, CRP has been recognised as a contentious marker with a previous review reporting vast conflicting findings (Michigan, Johnson & Master, 2011). A more recent meta-analysis has suggested that exercise is effective at reducing CRP concentration regardless of age (Fedewa, Hathaway & Ward-Ritacco, 2017). Previous studies have assessed CRP responses to pulmonary rehabilitation (Canavan et al., 2007; Sciriha et al., 2017) and an isolated acute bout of exercise (Spruit et al., 2007). Findings from these studies showed no effect which were supported by data presented in this thesis (Studies 2 & 4). It is also important to note that exercise responses of CRP in healthy populations has been conflicting with research suggesting that reductions in CRP following exercise training are only present when accompanied by a significant reduction of body weight or body fat (Choi, Joseph, Pilote, 2013).

This thesis poses the question of the suitability for assessment of CRP in the context of exercise in COPD. Studies 2 & 4 demonstrated the degree of variability between COPD patients in CRP concentrations further echoing issues found in previous research (Aksu et al., 2013). This was further confirmed in Study 5 where a pilot study in a post-pulmonary rehabilitation setting demonstrated the variability of CRP both between and within patients over time. Hence, based on the available evidence to date, systemic CRP may be of limited use in understanding the relationship between exercise, inflammation and exacerbations.

8.2.3.3 Total leukocytes and neutrophil counts

Total leukocyte and neutrophil count responses to exercise have been more widely explored in a healthy population (Pyne, 1994; Walsh et al., 2011), with limited

experimental evidence available in COPD (Jenkins et al., 2015; van Helvoort et al., 2005). A position statement suggested that exercise training programmes may need to be prolonged (Walsh et al., 2011), to have any effect on resting leukocyte and neutrophil counts and that an 8-week programme, similar in duration to that of previous research (Canavan et al., 2007; Sciriha et al., 2017), would not be sufficient enough to induce changes in these parameters. However, it was acknowledged that the COPD population suffer from chronic inflammation, in particular frequent exacerbators, whereby such parameters are significantly elevated compared to healthy individuals therefore creating a larger scope for reductions in these markers with exercise training. Interestingly, Study 2 for the first time showed that frequent exacerbators can experience significant reductions in total leukocyte and neutrophil counts. This is a novel finding suggesting that pulmonary rehabilitation has the potential to reduce differences in parameters of inflammation between frequent and infrequent exacerbators which may partly explain the reduction in risk of exacerbations with pulmonary rehabilitation (Moore et al., 2016). Study 4 highlighted the transient increases in circulating total leukocyte count and neutrophil count in response to acute exercise in COPD irrespective of training status (i.e. accustomed to exercise). This is in line with a large body of evidence in healthy individuals and hence simply reflects a typical physiological response to an acute stressor such as exercise. These transient changes are predominantly thought to be due to the demargination of cells from endothelial tissue or an inflammatory response to exercise-induced muscle damage (Brown et al., 2015; Pyne, 1994). Further research is required to determine the clinical implications of this modulation of inflammation.

8.2.3.4 Neutrophil activation markers

Study 2 & 4 adopted the novel approach of assessing responses of these neutrophil activation markers in response to exercise in frequent and infrequent exacerbators. Study 2 did not find any significant effects of pulmonary rehabilitation on neutrophil markers, but there was a tendency towards a significant increase in CD62L at the end of pulmonary rehabilitation. Based on previous evidence of the role of CD62L, it is conceivable to suggest that reduced neutrophil activation characterised by an increase in neutrophil CD62L expression (or rather a decreased shedding from the cell surface) is again indicative of an anti-inflammatory effect (Takahashi et al., 2013). It is also important to note that frequent exacerbators had an improvement in CD62L expression to the baseline levels observed in infrequent exacerbators suggesting a potential role of pulmonary rehabilitation in restoring inflammatory markers to 'normal' levels. In contrast, neutrophil CD11b and CD66b, which are upregulated when neutrophils are activated (Fortunati et al., 2009), were not affected further showing that pulmonary rehabilitation does not exacerbate resting inflammation. Study 4 suggested that CD62L was significantly decreased following an acute exercise bout, suggesting increased neutrophil activation (Takahashi et al., 2013), at the beginning of pulmonary rehabilitation in frequent exacerbators only, providing indications of differing immune responses to acute exercise between frequent and infrequent exacerbators. Non-significant increases in CD11b & CD66b were also observed post-exercise in frequent exacerbators further adding to the notion of an increased neutrophil activation post-exercise.

The utilisation of flow cytometry for the measuring of neutrophil activation came with numerous complications which is reflected by the small sample size. Firstly, flow cytometers comprise technical equipment including lasers and fluidics for the analysis of cellular populations using a high throughput (Ibrahim & van den Engh,

2007). Due to the multiple dependents (e.g. lasers, fluidics, vacuum) involved in the analysis of these cellular populations, the machine used for analysis in this thesis was susceptible to breakdown. Such limitations impacted on the sample size. Secondly, and a point that has wider significant importance were the issues surrounding the lack of expression of CD16b on neutrophils in the populations studied in this thesis (Figure 8.2). CD16 has been, and still is, considered a valid and important marker for identifying neutrophils (Lakschevitz et al., 2016). Manufacturers highly recommend the use of CD16 for the identification of neutrophils. There is very little in the literature to explain the phenomenon observed in subsets of COPD patients which was ruled out as a disease specific effect when similar observations were made in healthy smokers and healthy never smokers. Ultimately, this led to the withdrawal of these techniques for the follow-up study (Study 5). The assessment of literature suggested two potential reasons for the lack of expression of CD16b. One paper suggested a very rare hereditary deficiency of CD16 which could be due to genetic defects (Wagner & Hansch, 2004). Given the rarity, the current observations in this thesis would not plausibly align with these suggestions. A second observation was that elevated levels of ADAM17 can lead to the shedding of CD16b on neutrophils (Jing et al., 2015). ADAM17 has been suggested to be elevated in COPD (Stolarczyk & Scholte, 2018), potentially providing an area worthy of investigation, however similar observations in healthy comparators somewhat effect the clarity of this. This phenomenon of low CD16b was discussed with many leading experts in neutrophil biology with no conclusions for the underlying causes being reached. However, subsequent searching of unpublished sources (e.g. public research forums such as researchgate.net) identified that this issue is more widespread with several academics suggesting that CD16b may not be an advisable marker for the consistent identification of

neutrophils. Therefore, research or guidance is required around the suitability of CD16b as a marker to assess neutrophils. Studies 2 & 4 did suggest that neutrophil CD62L was a promising marker for further investigations with exercise in COPD. However, preliminary evidence presented in studies 2 & 4 suggest that there are minimal changes in neutrophil expression of CD11b and CD66b in the context of pulmonary rehabilitation.

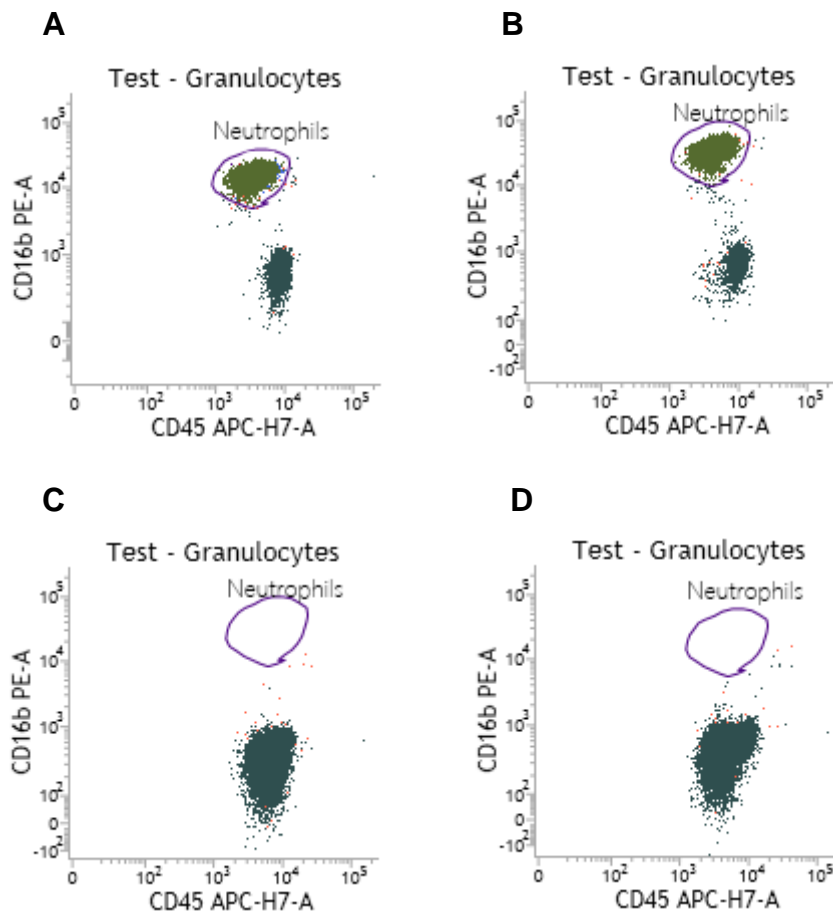


Figure 8.2. Example of a COPD patient pre- (A) and post-rehabilitation (B) with neutrophils identifiable by CD16b^{high}. Another example of a COPD patient pre- (C) and post-rehabilitation (D) with CD16b^{low} neutrophils. These examples were from analyses performed on the same day (for pre- and post-rehabilitation) utilising the same batch of antibodies for the given day.

8.2.3.5 Neutrophil maturity

The utilisation of the aforementioned neutrophil activation markers also allowed for the novel assessment of neutrophil maturity. This approach was adopted based on previous research identifying neutrophil subsets (Cortjens et al., 2017). This

approach was exploratory in nature and allowed for further insight into neutrophil dynamics and responses to exercise. For the first time, Studies 2 & 4 demonstrated the impact that acute and chronic exercise can have on neutrophil maturity in patients with COPD. In Study 2, pulmonary rehabilitation was shown to reduce the amount of progenitor neutrophils, a subset which is very immature in its function. Study 4 showed increases in immature neutrophils following an acute bout of exercise which has been previously observed with acute exercise in healthy individuals (Risoy et al., 2003). This further shows that exercise, in the context of pulmonary rehabilitation, has the potential to alter neutrophils in a similar pattern to healthy individuals. However, given the inflammatory state commonly observed with COPD, further research is required to confirm these responses with exercise, determining the clinical implications of such changes. Studies 2 & 4 demonstrate the potential of adopting the neutrophil phenotyping approach in exercise immunology.

8.2.3.6 Corticosteroid responses

When assessing immune functions in response to exercise, it is important to assess both pro- and anti-inflammatory mechanisms whereby exercise is considered to exert effects on both (Flynn, McFarlin & Markofski, 2007). It is important to establish a balance between pro- and anti-inflammatory responses to fully understand the effects of exercise on inflammation.

With this in mind, Study 3 adopted an exploratory and novel approach of assessing responses of anti-inflammatory genes. This approach involved the assessment of gene expression in PBMC's in response to treatment with a corticosteroid, a commonly used therapy in the treatment and management of COPD (Park, Man & Sin, 2012). The utilisation of this approach has never been adopted in the context of pulmonary rehabilitation and was based on previous research using an

established experimental model of assessing the effectiveness of corticosteroids in PBMC's and epithelial cells of COPD patients (Kelly et al., 2012; Newton & Holden, 2007). Study 3 demonstrated the potential effects that pulmonary rehabilitation may have on enhancing the action of corticosteroids in COPD by upregulating anti-inflammatory genes. The effects of non-pharmacological interventions, such as pulmonary rehabilitation, on the effectiveness of pharmacological treatments is an area vastly underexplored in all populations. The work undertaken on pharmacological therapies in pulmonary rehabilitation mainly revolves around bronchodilators whereby the use of these devices has been shown to assist with exercise training, allowing patients to train at higher exercise intensities (ZuWallack, 2008), and reduce dyspnoea during rehabilitation programmes (Maltais et al., 2005). Therefore, there is very little understanding with regards to the mechanistic effects on potentially enhancing the effects of pharmacological treatments. The findings presented in Study 3 should be investigated further utilising different cellular populations (e.g. airway epithelial cells) which may represent a more meaningful model of response to inhaled treatments (Ito et al., 2000). Future research should also assess a whole range of genes related to inflammatory mechanisms in COPD to determine further potential unknown anti-inflammatory effects of pulmonary rehabilitation, but these early findings certainly hold a lot of promise.

8.3 Daily respiratory symptoms

Study 1 demonstrated the ability of pulmonary rehabilitation to reduce daily respiratory symptoms (E-RS™) and recall of respiratory symptoms (CRQ). Within Study 4, attempts were also made to assess inflammatory responses to acute exercise alongside respiratory symptom monitoring to provide insights into the clinical impact of acute inflammatory responses to exercise in COPD. In healthy

populations, acute bouts of strenuous exercise have been observed to trigger elevations in respiratory symptoms (Walsh et al., 2011). In frequent and infrequent exacerbators of COPD, no changes were observed with respiratory symptoms following commencement of pulmonary rehabilitation, despite elevations in inflammatory markers following exercise. In fact, the respiratory symptoms almost appeared to improve following commencement of the pulmonary rehabilitation programme. This is of potential benefit as it could be argued that if elevated total leukocyte and neutrophil counts following exercise corresponded to increased respiratory symptoms, then this could increase the likelihood of a patient suffering from an exacerbation, which is a common factor for poor completion of pulmonary rehabilitation (Keating, Lee & Holland, 2011). These findings also appear to disagree with qualitative studies whereby patients have reported that they avoid exercise due to a fear of worsening their dyspnoea symptoms (Fischer et al., 2007; Harris, Hayter & Allender, 2008; Taylor et al., 2007; Young et al., 1999). However, the beginning of pulmonary rehabilitation tends to be of a lower intensity of exercise to 'ease' COPD patients into the programme, potentially explaining the findings presented in Study 4. Future research should make attempts to assess respiratory symptoms, over a period of days following a controlled acute bout of exercise, alongside inflammatory responses to determine the clinical relevance of short-term elevations in inflammatory markers.

8.4 Implications

8.4.1 Implications for practice

The overall aim of this thesis was to examine the role of the frequent exacerbator phenotype in pulmonary rehabilitation. The evidence presented in this thesis show that non-pharmacological treatments such as pulmonary rehabilitation have an important role in the COPD frequent exacerbator phenotype. This is characterised

by significant improvements in traditional clinical outcomes with pulmonary rehabilitation accompanied by reductions in inflammation. Some inflammatory mediators and clinical outcomes were also observed to be significantly improved in infrequent exacerbators showing that pulmonary rehabilitation should be inclusive to all COPD patients. However, the lower completion rates in frequent exacerbators is concerning and efforts should be made to target interventions towards tackling this.

The findings presented throughout this thesis may also result in the consideration of how pulmonary rehabilitation is implemented into COPD treatment pathways/algorithm. As expected, frequent exacerbators presenting to pulmonary rehabilitation met the criteria to be defined as GOLD grade D. Currently the treatment algorithm suggests triple therapy (LABA/ICS and LAMA) as a first-choice therapy for COPD patients in this category. Alternative options include combination therapies such as: LABA/LAMA, LABA/ICS and PDE4 inhibitor, or LAMA and PDE4 inhibitor (GOLD, 2019). Also, the majority of infrequent exacerbators presenting to pulmonary rehabilitation met the GOLD B criteria. Currently treatment guidelines suggest that the first line of treatment for these patients should be LABA or LAMA. The alternative option is the use of a combination of LABA and LAMA (GOLD, 2019). The NICE, (2018) guidelines suggest that pulmonary rehabilitation is a fundamental part of care and should be offered to patients who are breathless before starting inhaled therapies. The results presented in this thesis support these guidelines and suggest that anti-inflammatory benefits can be obtained with pulmonary rehabilitation supporting the need to repeat pulmonary rehabilitation in those who are most breathless and have recurrent exacerbations. This is particularly apparent in the GOLD D category, where anti-inflammatory treatments are considered to only be obtained from pharmacological therapies.

This thesis (Studies 1-4) was centred around a 'real-world' pulmonary rehabilitation intervention delivered to COPD patients as part of standard care. This intervention was not manipulated in any way by the design of these studies meaning that the findings represent evidence based on a standard clinical intervention which is commonly delivered as part of standard care. This has the potential to be of clinical relevance as opposed to attempting to apply findings from a study mimicking pulmonary rehabilitation but does not reflect a 'real world' setting. Resultantly, a wide variation of COPD patients, in terms of disease severity, were included in the studies comprising the make-up of this thesis meaning the findings are generalisable to other UK pulmonary rehabilitation services. Despite this, these studies did implement exclusion criteria to attempt to control for potential confounders suggest as other underlying inflammatory diseases which could influence the outcomes of the thesis.

In the literature review, a systematic review was also performed which has implications for practice. The findings of the review build upon those proposed by Moore et al. (2016) where pulmonary rehabilitation was shown to reduce the risk of hospitalisation. The review demonstrated the role supervised maintenance exercise programmes following pulmonary rehabilitation can have on enhancing the effect of reduced hospital admissions and exacerbations of COPD compared to that of pulmonary rehabilitation alone (Jenkins et al., 2018). These findings highlight the importance of providing/promoting longitudinal supervised exercise programmes for patients with COPD and efforts should be made to provide this as an effective way of exacerbation/hospital admission avoidance.

Practical guidance for the management of frequent exacerbators in the pulmonary rehabilitation setting –

- Pulmonary rehabilitation is equally, if not more important to frequent exacerbators and completion should be encouraged
- Ensure optimisation of pharmacological therapies and consider repeating pulmonary rehabilitation
- Reiterate the benefits of pulmonary rehabilitation in the management of COPD and exacerbations
- Improve self-management pathways and utilise physical activity interventions to encourage frequent exacerbators to reduce sedentary behaviours
- Provide/promote supervised maintenance options for COPD patients to continue exercising following pulmonary rehabilitation as an effective way of reducing the risk of exacerbation/hospital admission

8.4.2 Implications for research

The experimental evidence presented in this thesis brings numerous implications for consideration in future research. In Study 1, although completion rates were found to be significantly lower in frequent exacerbators of COPD, larger sample sizes are commonly required to provide confidence in this effect. Therefore, future larger scale studies, or audits of services, are required to confirm these findings. Confirmation of these findings could have significant implications on research and assessing the various phenotypes presented in pulmonary rehabilitation. The scope of this would result in research attempting to tailor interventions to specific subsets of patients (e.g. frequent exacerbators) to assist in completion.

Secondly, Studies 2 & 3 highlight the potential anti-inflammatory benefits, in terms of modulation of both pro- and anti-inflammatory mediators, of pulmonary rehabilitation on resting systemic inflammation (Table 8.1). With future research, the clinical impact of this could be of huge significance and have the potential to

change how pulmonary rehabilitation is marketed, which could indirectly have knock-on effects for completion rates. For example, if the efficacy and mechanisms of pulmonary rehabilitation on exacerbations or hospitalisations and enhancing pharmacological therapy are confirmed, this might be deemed a more attractive therapy for COPD patients and commissioners. Especially given that a recent paper found that COPD patients valued exacerbations as the most important outcome (Zhang et al., 2018). However, trials assessing inflammatory responses in the airways are also warranted to support observations seen in the systemic compartment.

It is too early to interpret the clinical effects of these systemic inflammatory responses to exercise in COPD. However, Study 4 was the first study in COPD to attempt the monitoring of daily respiratory symptoms and inflammatory responses to exercise in COPD (Table 8.1). This highlighted that respiratory symptoms did not appear to increase in response to inflammation with exercise. It has been suggested that exercise training may reduce the acute inflammatory response to exercise (Turton et al., 2002). This was not observed in Study 4 with similar inflammatory responses to exercise at the end of pulmonary rehabilitation compared to beginning. It is important to note that the exercise regimens were not controlled, and the progressive nature of the pulmonary rehabilitation programme may in part explain the lack of reduction in the acute inflammatory response to exercise. Future trials should assess acute inflammatory responses to exercise, in both the airway and systemic compartments, in COPD in a more controlled manner, but it is important to not undermine the evidence presented here which represents 'real-world' evidence within the context of a usual pulmonary rehabilitation programme. The continual assessment of respiratory symptoms alongside this is also encouraged if a correlation is to be established between acute inflammatory

responses to exercise and exacerbations. Establishing these links are important because if acute inflammatory responses to exercise in COPD are linked to exacerbations, then it will provide further emphasis on the decisions regarding the intensity of exercise used in pulmonary rehabilitation programmes. It is also important to note that the evidence presented in this thesis assessed markers of systemic inflammation, which are considered surrogate markers of exacerbation risk, as a key mechanism of the frequent exacerbator phenotype. However, whilst beyond the scope of this thesis, it would be prudent for future research to assess other mechanisms such as susceptibility to viral infections.

Table 8.1. Summary of inflammatory responses to chronic and acute exercise

Parameter	COPD patients		Frequent Exacerbators	
	Chronic	Acute	Chronic	Acute
Fibrinogen	↓	-	↓	-
CRP	-	-	-	-
Total leukocyte count	-	↑	↓	↑
Total neutrophil count	-	↑	↓	-
Neutrophil CD11b	-	-	-	-
Neutrophil CD62L	-	-	-	↓
Neutrophil CD66b	-	-	-	-
Mature neutrophils	-	-	-	-
Immature neutrophils	-	↑	-	↑
Suppressive neutrophils	-	-	-	-
Progenitor neutrophils	↓	-	↓	-
MKP-1	-	N/A	-	N/A
GILZ	-	N/A	-	N/A

Finally, Study 5 further highlighted the ever-increasing topic of interest in terms of the profile of COPD patients following completion of pulmonary rehabilitation. This pilot study showed that frequent exacerbators may adopt more inactive lifestyle habits. Despite this, it was unclear whether these inactive habits corresponded to an increase in systemic inflammation. There is a need for future research to assess physical activity in frequent exacerbators of COPD alongside inflammatory parameters suggested in this pilot study. This will help to determine whether frequent exacerbators need targeting with physical activity interventions.

Research priorities for exercise and inflammation in frequent and infrequent exacerbators of COPD –

- A large prospective cohort study of pulmonary rehabilitation compliance in frequent and infrequent exacerbators of COPD without limiting inclusion criteria (e.g. other co-morbid respiratory and/or inflammatory conditions).
- Designing and developing interventions tailored to help frequent exacerbators complete pulmonary rehabilitation.
- Further explore the effects of exercise programmes on systemic inflammation in COPD, utilising biomarkers such as fibrinogen, total leukocyte counts, neutrophil counts, neutrophil CD62L expression, and neutrophil phenotypes (mature, immature, suppressive, progenitor).
- Examine the effects of acute and chronic exercise on airway inflammation in comparison to systemic observations.
- Assess acute inflammatory responses to controlled exercise bouts in COPD, utilising total leukocyte and neutrophil counts, and determine whether progressive exercise training programmes (e.g. pulmonary rehabilitation) can dampen any acute inflammatory responses. It would be prudent to investigate daily respiratory symptoms alongside these inflammatory

responses to draw correlations between exercise-induced elevations in markers and patient reported symptoms.

- Further explore the effects (acute and chronic) of exercise training in COPD on the effectiveness of corticosteroids and other pharmacological therapies purported to have direct or synergistic effects on inflammation (e.g. LABA) utilising different genes (e.g. RGS family) and assessments at a protein level.
- Design interventions to target daily physical activity (e.g. step counts and time spent in light activity) and sedentary behaviour in frequent exacerbators.

8.5 Summary

In conclusion, this thesis provides convincing evidence that pulmonary rehabilitation provides clinical benefits to COPD patients with frequent exacerbations but they may require improved support in order to complete programmes or to be physically active post-rehabilitation. This thesis also provides the proof of concept that pulmonary rehabilitation can have anti-inflammatory effects in these patients. However, more research is needed to fully understand the frequent exacerbator phenotype and how inflammatory responses to exercise relate to respiratory symptoms and exacerbation frequency.

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Appendices

Appendix A: Systematic Review Protocol

Efficacy of supervised exercise following pulmonary rehabilitation on health status

Alex Jenkins, Holly Gowler, Ffion Curtis, Arwel Jones

Review question(s)

To assess the efficacy of continued supervised exercise training following completion of pulmonary rehabilitation on COPD-related health status

Searches

Searches of systematic review databases will be conducted; Cochrane, DARE and PROSPERO. The following databases will be searched for completed and ongoing trials: MEDLINE, EMBASE, CINAHL, Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science, ClinicalTrials.gov, PEDro, and Current Controlled Trials. We will also search the British Library (ETHOS) and Conference Papers Index as well as other library services within our institution for obtaining non-published data. Database searches will impose a restriction on articles translated into English, with searches encompassing all time points from inception to present.

Key search terms to be used to search trials registers and databases are terms related to chronic obstructive pulmonary disease (COPD), pulmonary rehabilitation, and exercise maintenance. Database searches are to be supplemented with internet searches (e.g. Google Scholar), contact with study

authors, experts and research groups, forward and backward citation tracking from included studies and systematic reviews.

Types of study to be included

Papers will only be included if they adhere to the following study designs:

Randomised Controlled Trials, Cluster Randomised Controlled Trials, Cross-over randomised trials (data up to point of cross-over only) or Quasi-randomised Controlled Trials.

Condition or domain being studied

Chronic Obstructive Pulmonary disease (COPD) is defined as a preventable and treatable disease characterised by airflow limitation that is not fully reversible (GOLD, 2010). COPD is now the third leading cause of mortality worldwide (British Thoracic Society, 2006). The main cause for hospitalisations in COPD is exacerbations of respiratory symptoms (World Health Organisation, 2012).

Participants / population

Patients who have completed pulmonary rehabilitation and taken part in supervised exercise training.

Intervention(s), exposure(s)

Trials to be included will have patients randomised to a supervised exercise training programme or any other form of care/non-exercise pathway following pulmonary rehabilitation.

Supervised exercise training will be defined as “supervised and prescribed exercise by an individual not partaking in the intervention”.

Exercise is defined as “a type of physical activity consisting of planned, structured and repetitive bodily movement done to improve or maintain one or more components of physical fitness. (Caspersen, Powell, & Christenson, 1985).”

Comparator(s) / control

Concurrent control group receiving any care post-pulmonary rehabilitation.

Outcome(s)

Primary outcomes

Hospital admissions (respiratory cause)

Exacerbations requiring treatment with short course of antibiotics or oral corticosteroids or both

Mortality

Secondary outcomes

Length of hospital stay

All-cause hospital admissions

Outpatient visits

GP visits

Data extraction, (selection and coding)

Selection of studies

Titles and abstracts will be independently screened by two reviewers in line with the inclusion criteria. Full text papers will be requested and assessed by two reviewers when studies are not excluded based on title or abstract. Any

discrepancies between the two reviewers will be resolved with the inclusion of a third reviewer.

Data extraction and management

Data extraction will take place using an adapted Cochrane Data Extraction Template including elements adapted from a Taxonomy form used previously (Lamb et al., 2011) for randomised controlled trials. Data extraction will be undertaken by one reviewer for each study, with cross checking taking place via a second reviewer. Papers will be compiled using Endnote referencing software.

The following study characteristics will be extracted:

Methods: Date/title of study, aim of study, study design, unit of allocation, duration of study, duration of intervention, primary outcome, other outcomes, and funding source.

Participants: Population description, demographics, inclusion criteria, exclusion criteria, method of recruitment of participants, total number randomised, clusters, baseline imbalances, withdrawal and exclusions, subgroups reported.

Intervention: Group name, number randomised to group-sample size, description, venue numbers/locations, duration and frequency of maintenance exercise training period, delivery, providers, co-interventions, compliance/adherence, and defined parameters of usual care.

Comparison: Usual care defined as a referral back to the patient's primary care provider for continued medical care with recommendations for post-pulmonary rehabilitation self-management at home.

Outcomes: Outcome name, outcome type, outcome definition, person measuring/reporting, unit of measurement, scales (upper and lower limits), is

outcome/tool validated?, imputation of missing data, assumed risk estimate, level of power.

One reviewer will independently analyse the extracted data using RevMan and then the data will be cross-checked by a second reviewer for accuracy purposes, yet again if there are any discrepancies in the reported data, a third party will be involved.

Risk of bias (quality) assessment

Assessment of risk bias in included studies

Two reviewers will independently assess risk of bias in included studies using the Cochrane Risk of Bias Table with the following domains: Random sequence generation, allocation concealment, blinding of participants, blinding of assessor, blinding of personnel, incomplete outcome data, selective outcome reporting, and other bias. These domains will be categorised as a high, low or unclear risk of bias. The Cochrane Risk of Bias assessment tool is designed to assess studies for accuracy of key quality issues such as randomisation, blinding and follow up. Each domain will be classified as adequate, unclear or inadequate with risk of bias for each study to be classified using the following criteria: (a) low risk of bias (all criteria are deemed adequate), (b) moderate risk of bias (one criterion graded as inadequate or two graded as unclear), and (c) high risk of bias (more than one criterion is deemed inadequate, or more than two graded unclear). Disagreements between reviewers over risk of bias in particular studies will be resolved through further discussion with the potential use of a third party. As it is not possible to blind participants to this intervention, the issue of blinding will only apply to outcome assessors and personnel.

Strategy for data synthesis

We will create narrative synthesis of the findings from the included studies, structured around the type of intervention, target population characteristics, type of outcome and intervention content. Due to a range of different outcomes measured and reported across the small amount of existing trials there may be a limited scope for meta-analysis. However, where studies overlap in terms of outcome measures, we will bring these data together using a random-effects meta-analysis, with mean differences for continuous outcomes, rates as rate ratios, and dichotomous data as risk ratios. Data will not be pooled if heterogeneity is moderate (I^2 statistic greater than 30%). If heterogeneity is identified, potential causes will be explored (e.g. clinical and/or methodological diversity). We will try to clarify heterogeneity via subgroup analysis, but if it cannot be explained (i.e. if there is considerable variation in the results, particularly inconsistency in the direction of the effect), then a narrative approach will be taken and a meta-analysis will not be performed.

Sensitivity analyses will be carried out on the included studies displaying a low risk of bias in accordance with the Cochrane Risk of Bias Table.

We plan to carry out subgroup analysis on the primary outcomes using the following:

- Supervised exercise alone versus supervised exercise and education
- Outpatient exercise vs. community exercise
- Frequency of exercise: <2 per week vs. ≥ 2 per week
- Supervised exercise compliance
- Impact of supervisor (e.g. qualifications)

Dissemination plans

This review is to be published in a peer-reviewed journal.

Appendix B: Systematic Review Supplementary Materials

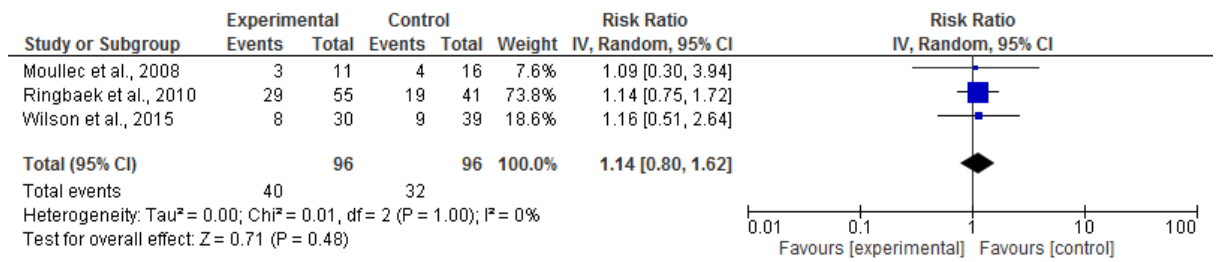


Figure S1. Trial-level data, effect estimates and forest plot of comparison for the risk of all-cause hospital admission.

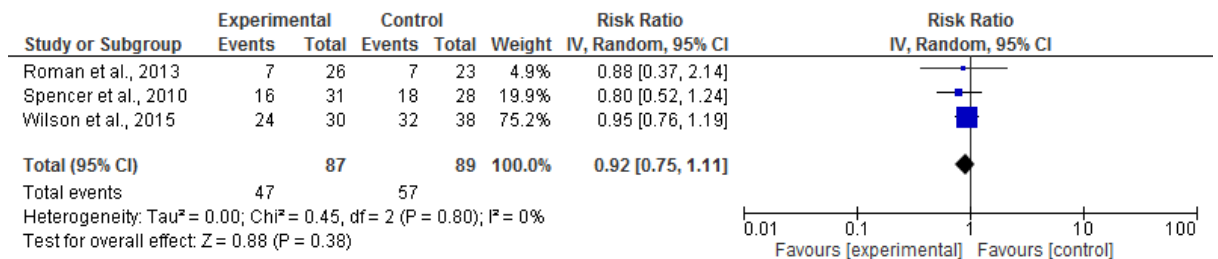


Figure S2. Trial-level data, effect estimates and forest plot of comparison for the risk of GP visits.

Table S1. Example search strategy of a bibliographic database (CINAHL)

	Search Term	Field
1	Lung diseases, obstructive	MH (explode)
2	Lung diseases, interstitial	MH (explode)
3	Pulmonary fibrosis	MH (explode)
4	COPD	TX
5	Chronic obstructive pulmonary disease	TX
6	COAD	TX
7	COBD	TX
8	emphysem*	TX
9	chronic bronchitis	TX
10	cystic fibrosis	TX
11	Pneumoconiosis	TX
12	Sarcoidosis	TX
13	Asthma	TX
14	Bronchiectasis	TX
15	Alveolitis	TX
16	Histiocytosis	TX
17	Granulomatosis	TX
18	Bagassosis	TX
19	asbestosis OR byssinosis OR siderosis OR silicosis OR berylliosis OR anthracosilicosis	TX
20	Scleroderma	TX
21	1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20	
22	Exercise therapy	MH (explode)
23	Activities of Daily Living	MH (explode)
24	Rehabilitation research	MH
25	Physical and rehabilitation medicine	MH (explode)
26	Physical fitness	MH
27	Exercise Movement Techniques	MH (explode)
28	Telerehabilitation	MH
29	Rehabilitation within 2 of: pulmonary OR respiratory OR physical OR early	TI, AB
30	exercis* within 3 of: supervi* OR training OR maintenance OR program*	TI, AB
31	physical activit*	TI, AB
32	Maintenance within 2 of: intervention OR group OR exercise OR program* OR training	TI, AB
33	22 OR 23 OR 24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 30 OR 31 OR 32	
34	Randomised	TI, AB
35	Randomized	TI, AB
36	Randomly	TI, AB
37	Trial	TI, AB
38	Controlled	TI, AB
39	34 OR 35 OR 36 OR 37 OR 38	
40	21 AND 33 AND 39	

Searches encompassed other chronic lung conditions as part of a wider review.